THE INFLUENCE OF MELATONIN ON THE ACTIVITY OF THE MAIN ENZYMES OF ANTIOXIDANT PROTECTION IN THE HEART OF RATS WITH DEXAMETHASONE DIABETES

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Summary

The goal of this study was to find out the influence of melatonin on the content of TBC-reactive products; on the activity of the main antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) in the heart of rats with dexamethasone diabetes.

Materials and Methods. The experimental study was carried out on thirty male eighteen-month-old non-linear white rats. The experimental animals were divided into three groups: 1) control (intact animals); 2) rats with diabetes; 3) rats to which, in addition to dexamethasone, melatonin in a dose of 10 mg per kg of body weight was administered intragastrically daily through a metal probe during the 13 days of experiment.

Results. In the heart of rats with dexamethasone diabetes, the content of TBC-reactive products increases by 43% compared with control rats, which indicates an increase in the processes of free radical oxidation of lipids. At the same time, in the heart of diabetic rats, a decrease in the activity of the studied enzymes is noted: superoxide dismutase – by 28%, catalase – by 25%, and glutathione peroxidase – by 31%, respectively, compared with control rats.

In the heart of rats, which, in addition to dexamethasone injections, received melatonin, the studied indicators did not differ reliably from the indicators of the control group of animals.

Conclusions. The use of melatonin stopped the processes of free radical oxidation of lipids and restored the activity of the investigated enzymes of antioxidant protection in the heart of rats.

Key words: Antioxidant, heart tissue, steroid diabetes, lipid peroxidation, experimental study.

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1. Introduction

Diabetes is the most common endocrine disease in the world. Currently, about 370 million people are suffering from this serious illness. Violation of the body's tolerance to glucose, the development of insulin resistance against the background of persistent hyperglycemia in type II diabetes is accompanied by an increase in the processes of free radical oxidation of lipids and biopolymers, inhibition of the functioning of antioxidant protection systems of tissues, in

particular the heart, and the development of oxidative stress. Therefore, it is advisable to use antioxidant agents in the complex therapy of diabetes *(Jia-Xu Li, 2022)*. Melatonin is known to be one of the powerful antioxidants *(Russel J Reiter, 2021)*.

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. Hyperglycemia can increase the indicators of lipid peroxidation and oxidative stress in which free radicals have the main role in the pathogenesis of these complications. Therefore, antioxidants which combat oxidative stress should be able to prevent and repair free radicals induced damages. Although free radicals contribute to kidney damage, atherosclerosis, diabetes, heart disease, nephrotoxicity and hepatotoxicity; however, clinical trials do not uniquely confirm a substantial impact on diabetic damage *(Rahimi-Madiseh M., 2016).*

The goal of this study was to find out the influence of melatonin on the contents of TBC-reactive products and oxidatively modified proteins; on the activity of the main antioxidant enzymes: superoxide dismutase (SOD), catalase and glutathione peroxidase (GP) in the heart of rats with dexamethasone diabetes.

2. Materials and methods

The experimental study was carried out on thirty male eighteen-month-old non-linear white rats. The experimental animals were divided into three groups: 1) control (intact animals); 2) rats with diabetes; 3) rats to which, in addition to dexamethasone, melatonin (Sigma, USA) in a dose of 10 mg/ kg was administered intragastrically daily during the experiment through a metal probe.

Diabetes was induced in rats according to the previously described method *(Stefanov O.V., 2001)*, by daily subcutaneous injection of dexamethasone at a dose of 0.125 mg/kg of the animal's body weight for 13 days. To induce the specified model of diabetes and the development of insulin resistance dexamethasone solution for injections – 4 mg/ml (KRKA, Slovenia) was used. Blood was taken from the tail vein to assess glycemia level using OneTouchUltra (LifeScan, USA). Euthanasia of animals was carried out in accordance with the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" *(Strasbourg, 1986)*.

Rat hearts were removed in the cold and used to prepare a 5% homogenate in 50 mM Tris-HCl buffer (pH=7.4). In centrifuge homogenates, the content of TBC-reactive products was determined *(Yury A.Vladimirov, 1995)* and the activity of the main enzymes of antioxidant protection according to well-known spectrophotometric methods: superoxide dismutase [EC 1.1.15.1] – according to the reaction of nitrotetrazolium reduction by superoxide radicals *(Elene E. Dubinina, 1994)*; catalase [EC 1.11.1.6] – due to the ability of hydrogen peroxide to form a stable colored complex with molybdenum salts *(Koroliuk M.A. et al., 1988)*; glutathione peroxidase [EC 1.11.1.9] – by the rate of oxidation of reduced glutathione *(Vlasova S.N., 1990)*. Determination of products of oxidatively modified proteins of neutral composition (OMP_{370nm}). In the process of oxidative modification of proteins, radicals of aliphatic amino acid residues form aldehyde and ketone groups, which react with 2,4-dinitrophenylhydrazine in an acidic medium to form colored 2,4-dinitrophenylhydrazones with a characteristic absorption spectrum *(Meshchyshen I. F., 1998).* The reliability of the difference between the obtained indicators was assessed using the parametric Student's t-test (for normal distribution) and the non-parametric Mann-Whitney U-test (for non-normal distribution). Differences were considered probable at $p \leq 0.05$.

3. Results

Melatonin injections caused a sharp decrease by 62% and normalization (on 13th day) in the elevated serum glucose level in diabetic group of rats compared with glucose level before treatment. This effect may be caused by the reason 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 *(Lin G.J., 2009)*. It is concluded *(Kushnir O.Yu., 2009)* that the hypoglycemic action of melatonin could be partly due to amelioration in the beta-cells of pancreatic islets.

The molecular structure of lipids makes them susceptible to oxidation. TBC-reactive substances are produced during lipid peroxidation *(Jesús Aguilar Diaz De Leon, 2020)*. In the heart of rats with dexamethasone diabetes, the contents of TBC-reactive products and OMP_{370nm} increase by 43% and 76% respectively compared with control rats, which indicates an increase in the processes of free radical oxidation of lipids and proteins.

SOD out-competes damaging reactions of superoxide, thus protecting the cell from superoxide toxicity. The reaction of superoxide with non-radicals is spin-forbidden. In biological systems, this means that its main reactions are with itself (dismutation) or with another biological radical such as nitric oxide (NO) or with a transition-series metal. The superoxide anion radical (O^{-2}) spontaneously dismutes to O_2 and hydrogen peroxide (H_2O_2) quite rapidly (~105 M−1s−1 at pH 7) *(Heinrich PC, 2006)*. SOD is necessary because superoxide reacts with sensitive and critical cellular targets. For example, it reacts with the NO radical, and makes toxic peroxynitrite. Superoxide inactivates the citric acid cycle enzyme aconitase, can poison energy metabolism, and releases potentially toxic iron *(Gardner PR, 1995)*.

We got the results, that in the heart of diabetic rats, a decrease in the activity of the studied enzymes is noted: superoxide dismutase – by 28%, catalase – by 25%, and glutathione peroxidase – by 31%, respectively, compared with control rats. These data have suggested that the increased production of oxygen reactive species in diabetes mellitus disorders might lead to a rapid consumption of antioxidants. In the levels of the antioxidant cascade, the decline in SOD activity means accumulation of reactive oxygen species, the significant decrease in catalase activity could indicate the failure to remove hydrogen peroxide, and the decrease of GP activity

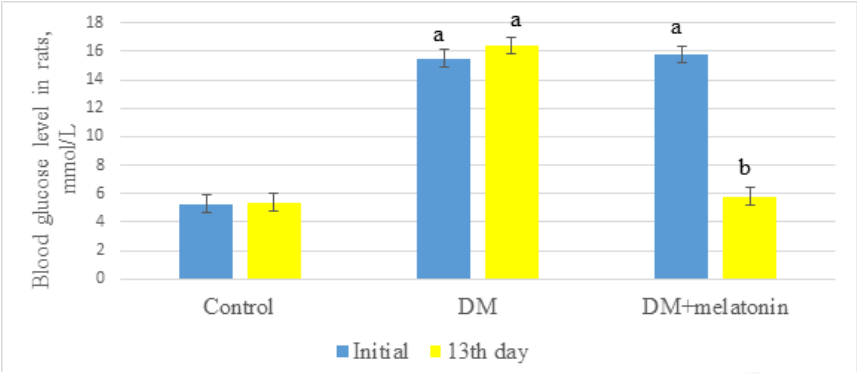

Fig. 1. Blood glucose level in rats, mmol/L (n = 10, x ± $S_{\bar{x}}$).

Note: 1. a, b, c – changes are reliable ($p \leq 0{,}05$).

2. a – concerning intact rats;

b – concerning rats with diabetes mellitus.

Table 1

Changes of the antioxidant defence in kidney of diabetic rats, (n=10, $x \pm S_{\bar{x}}$)

Indexes / Groups	TBC-reactive products, mkmol /g	OMP_{370nm}, nmol /g	SOD, U/mg	GP, nmol/min×mg	Catalase, mkmol/min×mg
1.Control group	34.2±2.05	2.21±0.097	0.54±0.049	236.3±9.42	164.9±10.53
2. DM	48.3±3.55[a]	3.04±0.122[a]	0.41±0.037[a]	185.0±17.8[a]	132.8±7.16[a]
3. DM + melatonin	39.5±2.95[b]	2.52±0.107[b]	0.52±0.044[b]	219.9±18.8[b]	149.2±12.3[b]

Note: 1. a, b – changes are reliable ($p \leq 0,05$).
2. a – concerning intact rats;
b – concerning rats with diabetes mellitus.

could also indicate the failure of the antioxidant system to produce enough reduced glutathione to avoid oxidative stress produced by free radicals. The malfunction of these enzymes may be related to the higher protein oxidation levels heart tissues of diabetic rats, which could affect the activity of theses enzymes.

The imbalance between reactive oxygen species production and the antioxidant defense, in favor of prooxidants, is causes oxidative stress. Although at physiological concentrations reactive oxygen species can function as signaling molecules regulating cell proliferation, growth, differentiation and apoptosis they react with and damage all classes of endogenous macromolecules including proteins, nucleic acids, lipids and carbohydrates *(Sadowska-Bartosz I, 2015)*.

As depleted, the antioxidant systems fail to protect the organism against the oxidative damage, so diabetic rats may have an inadequate antioxidant enzymatic activity that is unable to respond to increased free radical production, which could lead to the development of the complications. Diabetes may cause myocardial cell damage and eventually lead to the development of diabetic cardiomyopathy (DCM). DCM is a disease caused by diabetes that is independent of coronary artery disease, hypertension and heart valve disease. The main characteristics of DCM include oxidative stress, cardiac hypertrophy, apoptosis, myocardial fibrosis and impaired cardiac function *(Heather R., 2016)*.

In the heart of rats, which, in addition to dexamethasone injections, received melatonin, the studied indicators did not differ reliably from the indicators of the control group of animals. Melatonin is uncommonly effective in reducing oxidative stress under a remarkably large number of circumstances. It achieves this action via a variety of means: direct detoxification of reactive oxygen and reactive nitrogen species and indirectly by stimulating antioxidant enzymes while suppressing the activity of pro-oxidant enzymes. In addition to these well-described actions, melatonin also reportedly chelates transition metals, which are involved in the Fenton/ Haber-Weiss reactions; in doing so, melatonin reduces the formation of the devastatingly toxic hydroxyl radical resulting in the reduction of oxidative stress *(Russel J. Reiter, 2021)*. Possible, mealonin enhances the activities of SOD, catalase and GP which results in decresed of TBC-reactive products in heart tissues of diabetic rats.

4. Conclusions

Dexamethasone diabetes in the heart of rats is characterized by oxidant-antioxidant imbalance: the content of TBC-reactive products, oxidative modified proteins increase and the activities of the main enzymes of antioxidant protection – superoxide dismutase, catalase

and glutathione peroxidase – decrease. The use of melatonin stopped the processes of free radical oxidation of lipids and proteins, restored the activities of the investigated enzymes of antioxidant protection in the heart of rats.

References

1. Jia-Xu Li, Carolyn L. Cummins (2022) Fresh insights into glucocorticoid-induced diabetes mellitus and new therapeutic directions. Nature Public Health Emergency Collection, vol. 9, no. 18, pp. 540–557. doi: 10.1038/s41574-022-00683-6

2. Russel J. Reiter, Ramaswamy Sharma, Sergio Rosales-Corral Anti-Warburg Effect of Melatonin: A Proposed Mechanism to Explain its Inhibition of Multiple Diseases. Int J Mol Sci. 2021; 22(2): 764. doi: 10.3390/ijms22020764

3. 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):825-831. doi: 10.1016/j.apjtm.2016.07.001.

4. Stefanov, O. V. (Eds.) (2001). Doklinichni doslidzhennia likarskykh zasobiv. Kyiv: Avitsena.

5. Yury A. Vladimirov, Victor S. Sharov Elena S. Driomina Alexander V. Reznitchenko Sergey B. Gashev (1995). Coumarin derivatives enhance the chemiluminescence accompanying lipid peroxidation. Free Radical Biology and Medicine. Vol. 18, Issue 4, pp. 739–745. doi: org/10.1016/0891-5849(94)00199-T

6. Elene E. Dubinina Irina V. Shugaley Alexander T. Melenevsky Igor V. Tselinskii (1994). Characteristics of low molecular weight substances regulating plasma superoxide dismutase activity. Free Radical Biology and Medicine. Vol. 17, Issue 4, pp. 351–353.

7. Koroliuk M. A., et al. (1988) A method of determining catalase activity. Lab Delo. no. 1, pp. 16–19.

8. Vlasova SN, Shabunina EI, Pereslegina IA. (1990) The activity of the glutathione-dependent enzymes of erythrocytes in chronic liver diseases in children. Laboratornoe Delo, no. 8, pp.19–22.

9. Jesús Aguilar Diaz De Leon , Chad R Borges (2020) Evaluation of Oxidative Stress in Biological Samples Using the Thiobarbituric Acid Reactive Substances Assay. Journal of visualized experiments, vol.159, no.12:10.3791/61122. doi: 10.3791/61122

10. Heinrich PC, Löffler G, Petrifies PE (2006). Biochemie und Pathobiochemie (Springer-Lehrbuch) (German ed.). Berlin: Springer. p. 123. ISBN 978-3-540-32680-9

11. Gardner PR, Raineri I, Epstein LB, White CW (June 1995). "Superoxide radical and iron modulate aconitase activity in mammalian cells". The Journal of Biological Chemistry. 270 (22): 13399–13405. doi: 10.1074/jbc.270.22.13399. PMID 7768942

12. Kushnir A, Davydenko I. (2009). Influence of melatonin on condition of the langergans isles of the pancreas in alloxan diabetic rats. World of Medicine and Biology, vol. 4, no. 5, pp. 31–35.

13. Meshchyshen, I. F. (1998). Bukovynskyi medychnyi visnyk, 2 (1), 156–158.

14. Lin GJ, Huang SH, Chen YW, Hueng DY, Chien MW, Chia WT, Chang DM, Sytwu HK. (2009). Melatonin prolongs islet graft survival in diabetic NOD mice. Journal of Pineal Research, vol.3, no. 47, pp. 284-292. doi: 10.1111/j.1600-079X.2009.00712.x

15. Sadowska-Bartosz I, Bartosz G. (2015) Chapter 1: Oxidative nitrative and chlorinative stress: biomarkers, in: Studies on Psychiatric Disorders. Series: Oxidative Stress in Applied Basic Research and Clinical Practice. Humana Press, vol. XVIII, pp. 1–39.

16. Heather R. Cross, Lionel H. Opie, George K. Radda, Kieran Clarke (1996). Is a High Glycogen Content Beneficial or Detrimental to the Ischemic Rat Heart? Circulation Research, vol. 78, pp. 482–491.