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**Yaremii I.M.,
Kushnir O.Yu.,
Kharchenko A.O.**

Department of Bioorganic and Biological Chemistry and Clinical Biochemistry of the Higher education institution in Ukraine "Bukovinian State Medical University", Chernivtsi, Ukraine

CHANGES OF GLUTATHIONE SYSTEM PARAMETERS IN BLOOD OF ALLOXAN DIABETIC RATS UNDER THE INFLUENCE OF MELATONIN IN CONDITION OF LIGHT AROUND-THE-CLOCK

Abstract. *Insertion of melatonin for 7 days helped to reduce 1,7 times basal glucose level in the group of animals with overt diabetes in condition of light around-the-clock. Activity of glucose-6-phosphate dehydrogenase, glutathione peroxidase and glutathione reductase in the blood of rats with overt diabetes was on 45%, 32%, and 30% respectively lower than in control rats that were under artificial equinox. In rat blood with overt and latent diabetes occurred reduction of reduced glutathione content on 23% and 42% respectively compared with those of control. Insertion of melatonin to diabetic rats helped in normalization of parameters.*

Key words: *melatonin, alloxan diabetes, glutathione system, blood, rats, light around-the-clock.*

Introduction. Melatonin influences diabetes and associated metabolic disturbances. It acts as an antioxidant, neutralizing harmful oxidative radicals, and it is capable of activating certain antioxidant enzymes [3, 2]. It is a powerful antioxidant that easily crosses the cell membranes and blood-brain barrier [10, 17]. It acts as a direct scavenger of OH[•], O₂, and NO [12, 13].

The diabetogens, alloxan and streptozotocin, lead to selective destruction of beta-cells through their accumulation in these cells, where they induce the generation of ROS. Beta-cells are very susceptible to oxidative stress because they possess only low-antioxidative capacity. Results suggest that melatonin in pharmacological doses provides protection against ROS. Finally, melatonin levels in plasma, as well as the arylalkylamine-N-acetyltransferase (AANAT) activity, are lower in diabetic than in nondiabetic rats and humans. In contrast, in the pineal gland, the AANAT mRNA is increased and the insulin receptor mRNA is decreased, which indicates a close interrelationship between insulin and melatonin.

Oxidative stress plays a pivotal role in the development of diabetes complications, both

microvascular and cardiovascular [1, 16]. The increase in glycooxidation and lipoxidation products in plasma and tissue proteins suggests that oxidative stress is increased in diabetes [6, 9, 5].

Exogenous melatonin normalizes impaired due alloxan diabetes and tetrachlormethane hepatitis glucose-6-phosphatase activity in rat liver [19].

It has been ascertained [7] that an alloxan monohydrate administration to rats results in a significant elevation of the level of basal glycemia in the blood, and an increase of the activities of lactate dehydrogenase and glucose-6-phosphatase in the liver, however a decrease of the glycogen content and the activity glucose-6-phosphate dehydrogenase was in a direct dependence on the presence of hyperglycemia. The established changes of the indices of the carbohydrate metabolism in animals with alloxan diabetes turned out to be more marked under the conditions of permanent lighting than with equinox or permanent darkness. With a 7-day introduction of melatonin an improvement of the state of carbohydrate metabolism was marked and that was accompanied with a normalization of the indices, apart from the activities of glucose-6-phosphatase in the liver

which is normalized in case of a 42-day administration that was also characterized by a normalization of the level of glycosylated hemoglobin in the rat's blood [18].

Objective: to determine the influence of one-week melatonin infusion on basal levels of glucose, reduced glutathione (GSH), activity of glucose-6-phosphate dehydrogenase (G6PD), glutathione peroxidase (GPx) and glutathione reductase (GR) in the blood of alloxan diabetic rats under conditions of constant light.

Materials and methods. The experiments were carried out on 58 sexually mature male albino, not thoroughbred rats with the body mass – 0,18 – 0,20 kg. Alloxan diabetes was evoked via injecting the rats with a 5% solution of alloxan monohydrate intraperitoneally in a dose of 170 mg/kg following a 24 hour period of fasting [11]. The melatonin preparation was used in the research (the manufacturer – “Sigma”, USA). The animals were divided into 6 subgroups: 1) rats (the control group) that were under artificial equinox (Light : Darkness = 12 : 12); 2) rats that were under conditions of constant light (L : D = 24 : 0); 3) alloxan diabetic rats (L : D = 24 : 0); 4) alloxan diabetic animals which were introduced the melatonin preparation intraperitoneally in a dose of 10 mg/kg at 8 a. m. daily during 7 days starting with a 5-th 24 hour period after the injection of alloxan (L : D = 24 : 0); 5) alloxan diabetic rats with latent (basal glycemia < 6,9 mmol/l) diabetes (L : D = 24 : 0); 6) rats with latent diabetes which were introduced the melatonin preparation intraperitoneally in a dose of 10

mg/kg at 8 a. m. daily during 7 days starting with a 5-th 24 hour period after the injection of alloxan (L : D = 24 : 0). Blood was taken from the tail vein evaluate the BG level with the use of One Touch Ultra (LifeScan, USA). On the third day the death of a part (50%) of the alloxan diabetic animals was observed. Rats were sacrificed at the twelfth day of the experiment accordance with the ethical treatment of animals. Determinations of GSH, activity G6PD, GPx and GR in the blood were by standard methods [8]. Statistical analysis of results was conducted by Student's test. Sufficient level considered probability differences $r \leq 0,05$.

Results and discussion. Staying animals in lighting conditions around the clock throughout the week was accompanied by a tendency to increase in basal blood glucose by 18% from baseline this indicator on the 4-th day of the experiment (figure).

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

Probable reduction of melatonin synthesis and secretion under conditions of constant illumination coupled with reduced sensitivity to insulin, reduces the activity (table) of G6PD in control rats and rats with diabetes.

Under these conditions there was no typical increase in activity of G6PD in the group of animals with latent diabetes [18], but rather there was a decline of this indicator compared with those of control rats, provided equinox.

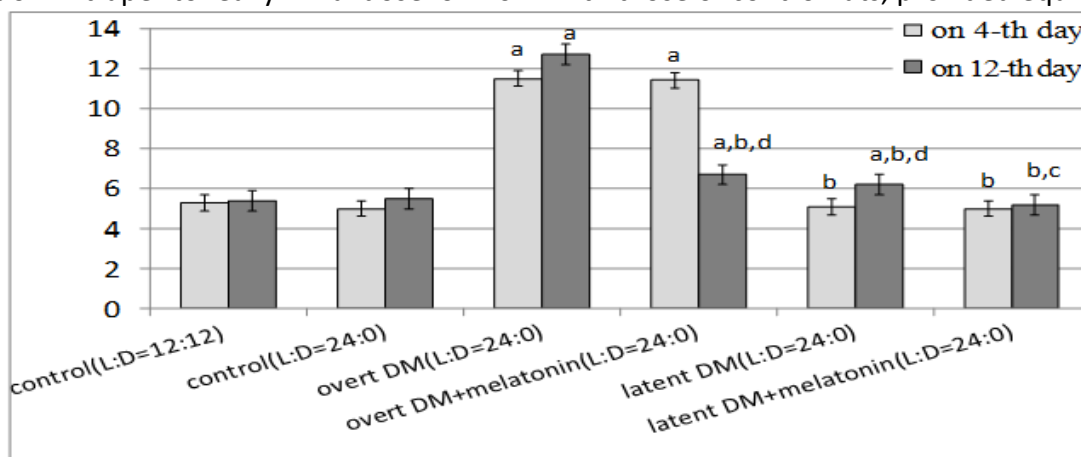


Figure. The level of basal glycemia (mmol/l) in blood of rats, ($n=6$, $\bar{x} \pm Sx$): 1. a, b, c - changes are reliable ($p \leq 0,05$). 2. a - concerning intact rats; b - concerning rats with overt diabetes; c - concerning rats with latent diabetes; d - concerning indices on 4-th day.

Table

Influence of melatonin on the indices of glutathione system in the blood of alloxan diabetic rats ($\bar{x} \pm Sx$, n=6)

Indexes	Glucose-6-phosphate dehydrogenase, mkmol/min×g Hb	Glutathione reductase, mkmol/min×g Hb	G-SH, mkmol/ml	Glutathione peroxidase, mkmol/min×g Hb
Control group (L:D = 12:12)	5,5±0,16	4,2±0,24	6,4±0,38	152,4±10,4
Control group (L:D = 24:0)	5,8±0,26	4,4±0,18	6,8±0,40	155,0±12,3
Overt diabetes (L:D = 24:0)	3,0±0,28 ^a	2,9±0,22 ^a	3,7±0,34 ^a	103,4±12,4 ^a
Overt diabetes+ melatonin (L:D=24:0)	5,6±0,45 ^b	4,4±0,28 ^b	6,6±0,42 ^b	155,2±11,0 ^b
Latent diabetes (L:D = 24:0)	5,0±0,44 ^b	3,7±0,26 ^b	4,9±0,40 ^{a,b}	140,6±12,6 ^b
Latent diabetes + melatonin(L:D=24:0)	6,2±0,27 ^{b,c}	4,5±0,24 ^{b,c}	6,8±0,44 ^{b,c}	162,4±11,0 ^b

1. a, b, c - changes are reliable ($p \leq 0,05$).
2. a - concerning intact rats ;
b - concerning rats with overt diabetes;
c – concerning rats with latent diabetes.

Activity of G6PD, GPx and GR in the blood of rats with overt diabetes was on 45%, 32%, and 30% respectively lower than in control rats that were under artificial equinox. In rat blood with overt and latent diabetes occurred reduction of reduced glutathione content on 23% and 42% respectively compared with those of control. We know [3, 4, 15] that pinealectomy, same as its hypofunction caused by permanent lighting, leading to decreased synthesis and secretion of melatonin, which causes insulin resistance and reduce the gene expression of glucose transporter GLUT 4, 2, 1. It is logical that the activity of G6PD is reduced under conditions of constant illumination during diabetes mellitus, whether an administration of melatonin leads to increased its activity.

Insertion of melatonin to diabetic rats helped in normalization of parameters that we studied.

Under the influence of melatonin increase activity of G6PD in the blood of rats may be due to the increasing number of substrate for G6PD (stimulating the flow of glucose into cells and its phosphorylation) and direct action [14].

According to our investigations the introduction of melatonin intraperitoneally in a dose of 10 mg/kg at 8 a. m. daily during 7 days to alloxan diabetic rats under conditions of constant light is conducive to a decrease in them of the level of fasting glucose, as well as – a stabilization of the indices of the body's antioxidant defense (glucose-6-phosphate dehydrogenase, glutathione peroxidase, glutathione reductase).

Conclusion. Under conditions of permanent light exogenous melatonin activates antioxidant glutathione dependent enzymes in the blood of alloxan diabetic rats that ultimately provides increased content of G-SH - one of the main endogenous antioxidant.

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