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Study of changes in the activity levels of purine nucleoside phosphorylase and the role of nitric oxide in the development of pathology in alloxan-diabetic rats

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Abstract

The problem of treating diabetes mellitus has not been resolved to this day; it requires a detailed study of various links of pathogenesis at the molecular level. In diabetes, conditions arise for the formation of oxidative stress. Diabetes mellitus is associated with the early development of cardiovascular complications, the immune system is destroyed. Endothelial cells produce nitric oxide (NO), a substance that is able to maintain a balance of vascular tone, coagulation and inflammation. Purine nucleoside phosphorylase (PNP) is one of the most important enzymes of purine metabolism, which characterizes the immune status of the organism.

The aim of this study was to study the enzymatic activity of PNP, as well as to determine the levels of nitric oxide in the blood serum, liver and pancreas in albino rats with simulated alloxan diabetes. The results of our studies on the detection of changes in the activity of PNP in the pancreas on the model of alloxandiabetic rats showed that at the late stage, the activity of PNP in the pancreas is completely suppressed, which indicates significant changes in the immune status of the body, the content of nitric oxide increases. Understanding the complex metabolic disorders that interact with the NO system may provide us with additional therapeutic options to reduce cardiovascular morbidity and mortality in diabetes mellitus. Indicators of NO and PNP can be used as additional paraclinical indicators for early diagnosis and to identify and clarify the degree of activity of the pathological process, the nature of the course, the stage of the disease, which contributes to the appointment of individualized adequate therapy.

Keywords: Alloxan diabetes; Nitric oxide; Purine nucleoside phosphorylase; Oxidative stress

1. Introduction

Diabetes mellitus (DM) is a global and one of the most acute health problems. The immune system, which has a certain autonomy in recognizing and removing foreign cells, antigens and other substances, at the same time is under strict homeostatic control, in which many biochemical reactions take part. And, since the problem of diabetes mellitus treatment has not been solved to this day, it requires a detailed study of various links of pathogenesis at the molecular level. Considering that the connection of immune processes with enzymes of purine metabolism is indisputable, it is of interest to study changes in purine metabolism [1,2]. In previous studies, a frequent combination and interrelation of purine and carbohydrate metabolism was noted [3].

Purine nucleoside phosphorylase (PNP) is one of the most important enzymes of purine metabolism that characterizes the immune status of the body. Inhibition of this enzyme leads to disruption of nucleoside homeostasis, which causes T-cell immunodeficiency [4]. In addition, as shown in one of the large-scale joint international projects on the use of

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proteomic methods for the identification of markers of hepatotoxicity induced by various agents, PFP was identified together with vitamin D-binding protein, malate dehydrogenase, paraoxanase, cellular retinol-binding protein and F-protein, one of the six earliest and most effective serum markers of hepatotoxicity [5]. Diabetes mellitus is associated with the early development of cardiovascular complications. Under physiological conditions, the endothelium protects against the development of atherosclerosis. Endothelial cells produce, for example, nitric oxide (NO), a substance that is able to maintain a balance of vascular tone, blood clotting and inflammation.

In DM, conditions arise for the formation of oxidative stress: the content of oxidation substrates (glucose and lipids) increases, the formation and activity of natural antioxidant systems such as glutathione, superoxide dismutase, catalase and glutathione peroxidase decreases [10,11].

The aim of this study was to study the enzymatic activity of PFP, as well as to determine the levels of nitric oxide in serum, liver and pancreas in white rats with simulated alloxan diabetes.

2. Material and methods

The study was carried out on mongrel white rats weighing 170-200 g. The rats were kept under controlled conditions according to Protocol #05072021/1; 05.07.2021 issued by the Committee of the Institute of Molecular Biology of the National Academy of Sciences of the Republic of Armenia for the Care and Maintenance of Animals. The rats were divided into 3 groups of 6 animals in each group. The number of animals was selected and calculated as $n = 6$, which corresponds to the minimum number of animals required to conduct an adequate and well-controlled study to perform an accurate and reliable statistical analysis of the data obtained.

All rats were anesthetized by intraperitoneal administration of a mixture of MMB (0.3 mg / kg body weight of medetomidine, 4 mg /kg of midazolam, 5 mg /kg of butorphanol). Adequate depth of anesthesia was provided by testing of paw retraction and palpebral reflexes. All rats were killed by an overdose of carbon dioxide, followed by blood sampling from the carotid artery and subsequent decapitation.

Alloxan diabetes (AD) was caused by the administration of 40 mg/ kg of alloxan. The glucose level in capillary blood was determined using a glucose meter on the 10th, 15th and 20th days after the administration of alloxan. Animals with a sugar content above 11-14 mmol/l were taken into the experiment.

After decapitation, blood samples were obtained and organs – the liver and pancreas - were extracted for analysis. Blood after coagulation was centrifuged in a refrigerated centrifuge at 12 thousand rpm for 30 minutes and the resulting blood serum was used on the same day to determine the activity of the enzyme. The pancreas and liver were washed from the blood with a cooled saline solution, homogenized in an extraction solution of 0.1M tris-HCl pH 7.2 containing 5mM DTT and 1mM EDTA and the resulting organ extracts were centrifuged at 18 thousand rpm for 30 minutes. Further, the homogenates (supraventricular fluid) were used to determine the activity of the enzyme. The activity of PF was determined by the accumulation of guanine, the amount of which was determined by a color reaction with Folin reagent and expressed in mmol of the substrate used per 1 g of wet tissue per minute, and for blood serum – mmol / l/min. The duration of incubation in a water thermostat at 37oC to determine the enzymatic activity was 6 minutes. The measurements were carried out spectrophotometrically using quartz cuvettes with a run length of 1 cm, at a wavelength of 750nm [5].

The final product of nitric oxide (NO) was determined using Grace reagent (1% sulfonamide, 0.1% naphthylendiamine, 2.5% phosphoric acid), the absorption of the solution was measured at a wavelength of 546 nm. Sodium nitrite was used as a standard [14].

For statistical data processing, the SPSS package (Statistical Package for Social Science) was used. The nature of the distribution of the obtained data was determined by the Kolmogorov-Smirnov criterion. Comparative analysis was performed using the nonparametric Mann-Whitney test. The differences were considered significant at $p \leq 0.05$ and $p \leq 0.01$. A refrigerated centrifuge, a spectrophotometer LKB Biochrom ULTROSPECII (Sweden), a pH meter PL-600 from mrc (Israel), guanosine and ATP from Sigma were used in the work.

3. Results and discussion

According to the results of our studies, it can be seen that with alloxan-induced diabetes mellitus, there is a significant decrease in the body weight of experimental animals and an increase in daily water intake (Table 2). Also, according to our observations, the largest volume of blood glucose was observed on day 20 (Table 1).

Table 1 Dynamics of blood glucose levels in experimental alloxan diabetes

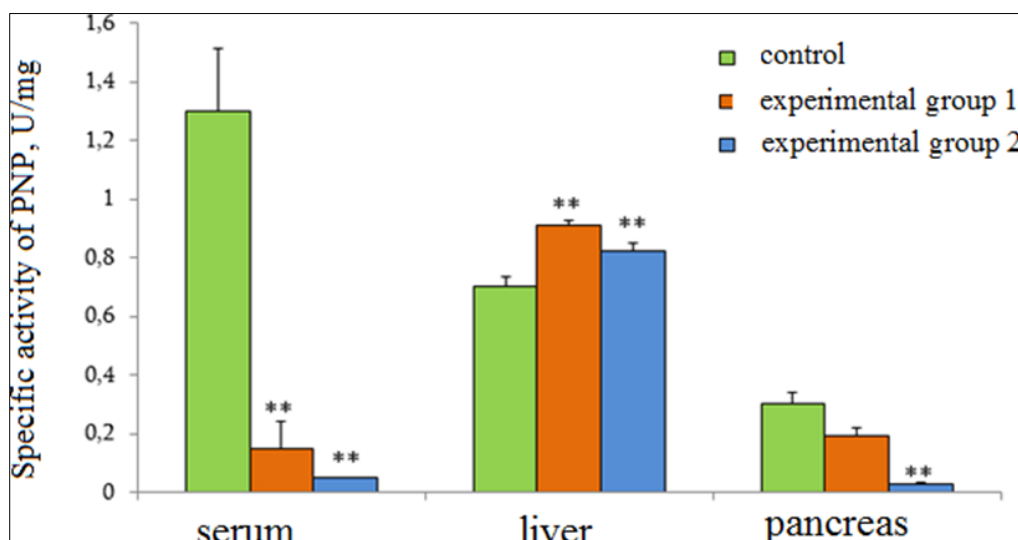
Experimental groups	Blood glucose level (mmol/L)		
	Days of the experiment		
	1	10	20
Control	3.8 ± 0.2	4.03 ± 0.4	3.9 ± 0.03
Experimental group	5.8 ± 0.3*	8.3±0.4*	12.2 ± 0.5*
	p=0.00035	p=0.000035	p=0

Table 2 Dynamics of physiological parameters of laboratory animals with alloxan diabetes

Physiological indicators	Experimental groups	
	Control group	DM group
Body weight (g)	250 ± 10	180 ± 6*
		p=0.000202
Water consumption (ml/day)	20 ± 3	32 ± 4*
		p=0.0398

Note: *p < 0.05 — the reliability of differences when comparing the indicators of the experimental groups with the control ones. In each group n=6.

As the results of studies have shown (Fig.1), there are significant differences in the activity of the enzyme between the studied organs, liver and pancreas, but there is also a similarity. It consists in suppressing the activity of PNF in the second experimental groups of all organs.



Note: ** - the difference from the control is significant at p ≤ 0.01, in each group n =6.

Figure 1 Dynamics of changes in the level of PFP activity in blood serum, liver and pancreas in both experimental groups: experimental group 1 - 10 days after the introduction of alloxan, experimental group 2 - 20 days after the introduction of alloxan

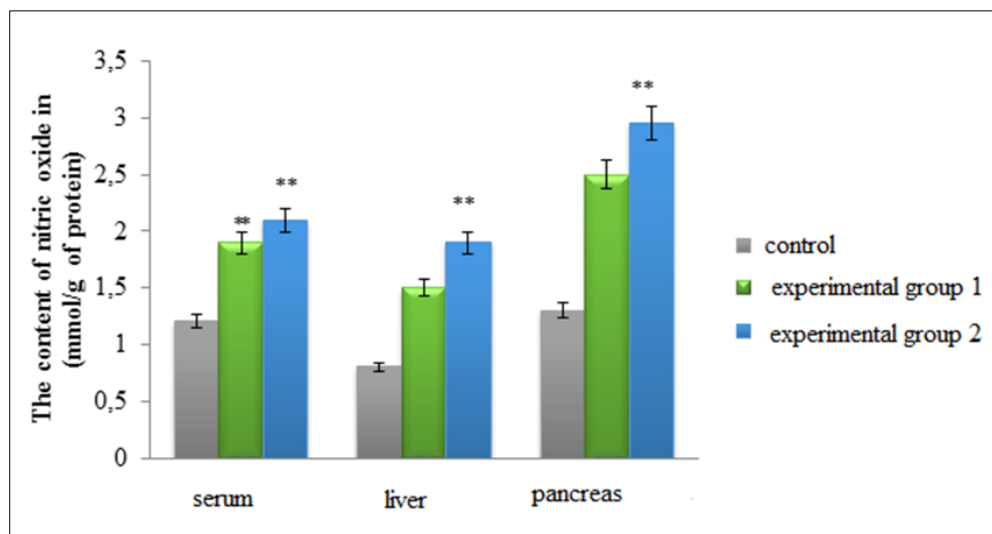
In our previous work, studying the influence of various environmental factors on the activity of PFP in some organs of rats, we revealed a different degree of sensitivity of the studied organs, assessing the values of enzyme activity on changing environmental conditions. Blood serum was the most affected, and hepatic PF showed resistant properties [6,8].

In the same work, the activity of PFP in all the selected organs varied in different ways. In the diagram, we see a sharp decrease in the level of enzyme activity in blood serum compared to the control in the 1st experimental group and almost complete suppression of activity in the 2nd (Fig.1). Such a steady decrease in the activity of PF is characteristic of a number of pathologies [1]. In addition, it cannot be excluded that a decrease in the activity level of the serum enzyme may be associated with damage to the shaped elements of the blood (especially erythrocytes), characterized by a high content of PFP [4].

In contrast to the blood serum, the values of the activity levels of PFP in the liver in both experimental groups increased. And although the activity in the 2nd experimental group decreases somewhat relative to the 1st, nevertheless it remains elevated compared to the control group of animals. It is well known that the liver plays a vital role in the synthesis and metabolism of carbohydrates, lipids, proteins, i.e. it has a very wide functional and metabolic spectrum. The organ also consists of hepatocytes, in which the greatest activity of PF is detected. Therefore, when diabetes mellitus fails in the metabolism and synthesis of glucose, we can only assume that the values of the activity of PF will increase.

The pancreas (pancreas) is a unique organ with both exocrine and endocrine functions. To detect violations of the exocrine function of the pancreas, various methods are used, including the determination of enzyme activity or the assessment of the degree of cleavage of the substrate by the enzymes of the pancreas [7]. The role of immunological studies to assess the severity of secondary immunodeficiency in various diseases of the pancreas has increased. The results of studies of immune parameters in such patients testified to various deviations in the immune system, which means that the mechanisms of natural immunoresistance were weakened. Hyperglycemia, as a consequence of diabetes mellitus, causes severe damage to various tissues, and consequently changes in the activity of enzymes in these tissues [9].

The results of our studies on the detection of changes in the activity of PF in the pancreas on the model of alloxandiabetic rats showed that at the late stage of the disease (in our experiment, it is 20 days after the introduction of alloxan) in the 2nd experimental group of rats, the activity of PF in the pancreas is completely suppressed, which indicates significant changes in the immune status of the body. As shown by the results of our studies in alloxandiabetic rats on the 10th and 20th days in the blood serum, the content of nitric oxide in the pancreatic and liver tissues increases in both experimental groups with the development of oxidative stress.(figure 2)



Note: ** - the difference from the control is significant at $p < 0.05$. In each group $n=6$.

Figure 2 The content of nitric oxide in (nmol / g of protein) in blood serum, liver and pancreas in both experimental groups: experimental group 1 – 10 days after the introduction of alloxan, experimental group 2 - 20 days after the introduction of alloxan

Under oxidative stress, reactive oxygen species (ROS) are formed – unstable and extremely reactive metabolites [10,11]. When nitric oxide (NO) interacts with the superoxide anion, the active compounds nitrosonium (NO⁺), nitroxyl (NO⁻) and peroxynitrite (ONOO) are formed. At elevated concentrations of NO (>1 mM), it interacts with respiratory chain complexes (cytochrome oxidase, ubiquinone), leading to inhibition of adenosine triphosphate synthesis [12]. The targets of the direct action of NO are copper and zinc atoms, which are part of enzymes. It has been established that nitrogen oxide, which is formed in the islets and β -cells of the pancreas, plays an important role in the mechanisms of destruction and death of β -cells, which leads to a sharp decrease in their number and the development of DM [13]. Diabetes mellitus is associated with the early development of cardiovascular complications. Under physiological conditions, the endothelium protects against the development of atherosclerosis. Endothelial cells produce nitric oxide (NO), a substance that is able to maintain a balance of vascular tone, coagulation and inflammation. However, in pathological conditions, for example, in diabetes mellitus, there may be a violation of NO-synthase (NOS) activity, which may be caused by a violation of NO production due to disconnection of receptor-mediated signal transmission, deficiency of L-arginine, NO-synthase substrate (NOS), or a decrease in the availability of one or more cofactors necessary for optimal functioning. However, hyperglycemia also stimulates the production of glycosylation end products and activates protein kinase C. These conditions can lead to increased oxidative stress. Reactive oxygen species rapidly inactivate NO, which leads to the formation of peroxynitrite. Peroxynitrite is a toxic oxidizer capable of damaging many biological molecules. A decrease in the availability of NO may be related not only to the development of atherosclerotic complications in diabetes, but may also disrupt postprandial glucose utilization mediated by insulin, and possibly contribute to the development of insulin resistance. Understanding complex metabolic disorders interacting with the NO system may provide us with additional therapeutic opportunities to reduce cardiovascular morbidity and mortality in diabetes mellitus.

4. Conclusion

The obtained results demonstrated statistically significant changes in the indicators of the level of PFP activity in the organs and stages of the disease studied by us. At the late stage of the disease, the activity of PF in the pancreas is completely suppressed, which indicates significant changes in the immune status of the body and may contribute to the development of immunoresistance. As the results of our studies have shown in alloxandiabetic rats on the 10th and 20th days in blood plasma, the content of nitric oxide increases in the tissues of the pancreas and liver with the development of oxidative stress, contributing to the development of insulin resistance. NO and PF indicators can be used as additional paraclinical indicators for early diagnosis and to identify and clarify the degree of activity of the pathological process, the nature of the course, the stage of the disease, which contributes to the appointment of individualized adequate therapy.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

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