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OBSAH

CHANGES IN THE INTERSTITIAL RENAL TISSUE OF THE EXPERIMENTAL ANIMALS IN DIABETES MELLITUS MODELLING

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***Annotation**. The article deals with findings obtained by histochemical study of changes in protein amines. A new results were received after applying a proper technique (by Mikel Calvo) of kidney histological sections staining in experimental animals in the early stages of streptozotocin-induced diabetes. It was noticed that in the early stages of experimental diabetes, lesions in protein amines of rats' renal glomeruli occures in various sequential order. The most essential changes were revieled on the 21st day of the experiment. This period is considered to be a time of adaptation of the kidneys to the influence of chemical substance – streptozotocin.*

***Keywords:** kidneys, streptozotocin, staining, amines, diabetes mellitus.*

Introduction. Diabetes mellitus and its serious complication – diabetic nephropathy – is a world leader among the causes of terminal renal failure [1, 4, 5]. This disease affects the renal vessels, arteries, renal tubules and glomeruli. Kidney malfunction occurs due to changes in the metabolism of lipids and carbohydrates.

Two basic pathophysiological mechanisms by which hyperglycemia leads to a damage of organs and tissues have been considered recently. The first of them is based on increasing intracellular glucose, leading to a steady metabolism disorder and development of generalized endothelial dysfunction as well as to the enhanced synthesis of type 4 collagen, laminin and fibronectin by the mesangial cells. The accumulation of these compounds leads to the development of glomerulopathy and thickening of the glomerular basement membrane [7, 8].

The second mechanism of the tissue damage is based on the processes of non-enzymatic glycation of proteins in the body. Part of the excess glucose in chronic hyperglycemia binds to free amino acids of circulating or tissue proteins. This non-enzymatic process produces reversible early glycation products, and later, irreversible advanced glycation end products (AGEs), which accumulate in the tissues and contribute to the development of microvascular complications of diabetes mellitus [6, 9, 10, 16].

Glycation of structural proteins such as collagen, elastin and myelin is pathogenetically more important. In this case, keto amines, formed after the intramolecular reconstruction of the schiff bases, accumulate in structural proteins and lead to the formation of the advanced glycation end products (AGEs) [11, 13,15].

In particular, in the kidneys AGEs, which were formed in the glomerular basement membrane, fix such proteins as albumin and IgG on it, which leads to the thickening of the basement membrane and changes in the structure, functions and architectonics of the tissue proteins. It results in changing the structure and properties of the renal glomeruli,

their basement membrane as well as their vascular wall.

AGEs receptors are found in the membranes of monocytes, macrophages, on the endothelial ones as well as on those of mesangial cells. The activation of these receptors leads to an increased production of cytokines, growth factors, which play an important role in the formation of pathological processes in diabetes mellitus [11, 12, 14].

Despite the significance of diabetic nephropathy, there is still incomplete understanding of the pathogenic mechanisms, particularly those underlying the differential susceptibility to this disease.

Objective. To determine quantitative parameters of the ratio between the amino and carboxyl groups of proteins in different structures of the renal glomeruli of experimental rats in early stages of drug-induced diabetes using a histochemical technique.

Material and methods. The experiment was conducted on 30 male mature nonlinear white rats, weighing 0.17 - 0.20 kg. The animals were divided into four groups. The first (I) - control group (n = 7) was on the standard mode of feeding, lighting and maintenance. The experimental groups of animals (II-n = 8; III-n = 8 and IV-n = 7) were administered streptozotocin (Sigma, USA) once intraperitoneally at a dose of 70 mg/kg [2]. In the second group the animals were slaughtered and studied 11 days after streptozotocin administration, the performance of the animals of the third group was studied after 21 days and in the fourth group after 31 days, respectively. Blood glucose was determined by measuring it with a glucose meter; the animals with the reliable glucose rate no lower than 10 millimole/L were selected for the experiment [2].

To study the key indices, the animals were slaughtered under light ether anesthesia, adhering to the provisions of Directive EEC №609 (1986) and MHP of Ukraine №690 of 23.09.2009. «On measures for further improvement of the organizational rules of using experimental animals.»

Computer microspectrophotometry method was implemented with all standardization requirements. The digital copies of images obtained by means of the microscope Delta Optical Evolution 100 (plan achromatic lenses) and digital camera Olympus SP-550UZ were analyzed with the computer software Image J (1.48v, free license, W. Rasband, National Institute of Health, USA, 2015) [3], in particular, using the probe method by analyzing color RGB system, we received the values «R» and «B», which served as a base to calculate R / B ratio. We also calculated the arithmetic mean and its error, and the differences in the average trends were evaluated using unpaired Student's t test (computer software PAST 3.10, free license, O.Hammer, 2015) [3].

The ratio R / B is interpreted as follows: if it is more than «1», it means that the carboxyl groups dominate over the amino groups in the proteins, and the greater this value, the more substantial predominance. If the value of ratio R / B is less than «1», then the amino groups prevail over the carboxyl groups in the proteins. The value of this index is in the fact that during the entire period of diabetes development the processes of oxidation and glycation of the protein amino groups get initially activated, which leads to changes in the ratio between carboxyl groups and amino groups in the proteins.

Results and discussion. Taking into account the fact that diabetes mellitus is

a disease that is characterized by the development of angiopathy, our attention was, first of all, drawn to the subendothelial basement membrane of the blood vessels in the interstices of the cortex and medullary substance and in the renal papilla. Unlike the renal glomerular capillaries, it was quite possible to differentiate the endothelium and basement membrane in the blood vessels of small and medium caliber in the interstices of the cortex, medullary substance and renal papilla in most studies. Therefore, the endothelium and subendothelial basement membrane of the blood vessels in these sites were examined separately.

The findings on subendothelial basement membranes of the blood vessels in the interstices of the cortex and medullary substance as well as in the renal papilla are shown in Table 1. One can see the most considerable changes in the third experimental group – R/B ratio is 1,29±0,016, which is 24% more than in experimental groups I (1,04±0,010) and II (1,04±0,014). The last experimental group has shown the result 1,31±0,017, which is almost 26% more than in intact animals, but does not show reliable difference comparing to the previous groups.

Table 1

Ratio R / B in the subendothelial basement membranes of the blood vessels in the interstices of the cortex and medullary substance and in the renal papilla in drug-induced diabetes at different time of the experiment (X±sx)

Group name	Ratio R/B	Probability of differences (P)
1. Experimental group I Intact animals (n=7)	1,04±0,010	
2. Experimental group II (n=8)	1,04±0,014	Pi>0,05
3. Experimental group III (n=8)	1,29±0,016	Pi<0,001 P11<0,001
4. Experimental group IV (n=7)	1,31±0,017	Pi<0,001 P11<0,001 P21>0,05

Note. Pi – probability of differences from intact animals, P11-probability of differences from intact animals on the 11th day, P21 - probability of differences from intact animals on the 21st day (by Mann-Whitney criterion).

The following findings were obtained for the R/B ratio in endothelial cells: on the 11th day of the diabetes (experimental group II) this coefficient was 1,16±0,019, on the 21st day of the experiment it was 1,40±0,016 and on the 31st - 1,44±0,017 respectively (Table 2).

Table 2

Ratio R / B in the endothelial cells in the interstices of the cortex and medullary substance and in the renal papilla in drug-induced diabetes at different time of the experiment (X±sx)

Group name	Ratio R/B	Probability of differences (P)
1. Experimental group I Intact animals (n=7)	1,14±0,017	
2. Experimental group II (n=8)	1,16±0,019	Pi>0,05
3. Experimental group III (n=8)	1,40±0,016	Pi<0,001 P11<0,001
4. Experimental group IV (n=7)	1,44±0,017	Pi<0,001 P11<0,001 P21>0,05

Note. Pi – probability of differences from intact animals, P11-probability of differences from intact animals on the 11th day, P21 - probability of differences from intact animals on the 21st day (by Mann-Whitney criterion).

The figures presented in tables 1 and 2 indicate that both the subendothelial basement membranes and the endothelium of blood vessels in the interstices of cortex and medullary substance as well as in the renal papilla responded in terms like subendothelial basement membranes and the endothelium of the kidney glomeruli, namely, on the 21st day of the experiment (Fig 1).

Conclusion. In streptozotocin-induced diabetes, an affection of amino groups of proteins in the renal glomeruli occurs on the basement membranes of the kidney glomeruli vessels with endothelial cells on the 21st day of our study.

Prospects for further research. The results of the study open perspectives for further research on histochemical features of oxidative modification of proteins in the cells of the renal glomerulus in the early stages of experimental streptozotocin-induced diabetes in rats.

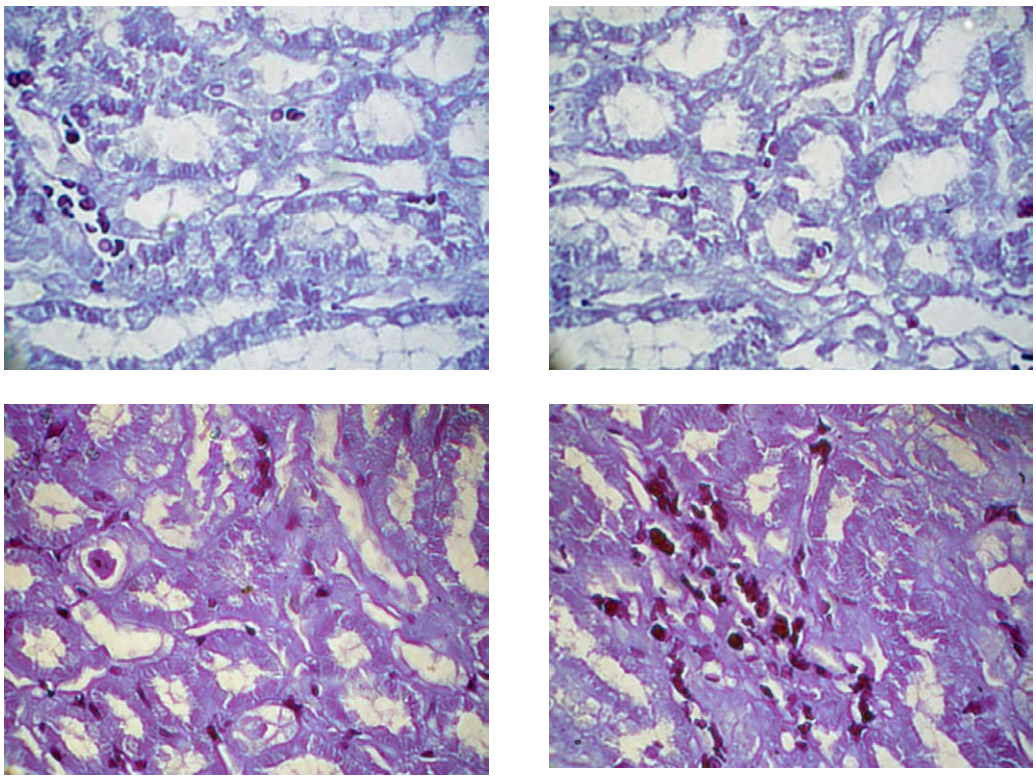

Fig. 1. Kidney medullary substance.
A) Intact animal;
B) Experimental diabetes mellitus on the 11th day;
C) Experimental diabetes mellitus on the 21st day;
D) Experimental diabetes mellitus on the 31st day;
The staining of histological sections by Mikel Calvo technique. Obj.40x.

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