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OBSAH

Lékařství a fyziologie

THE VIOLATION OF CERTAIN STRUCTURES OF RATS' RENAL GLOMERULI IN DRUG-INDUCED DIABETUS MELLITUS USING A HISTOCHEMICAL TECHNIQUE

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Annotation. *The article deals with findings obtained by histochemical study of changes in protein amines after applying various techniques of kidney histological sections staining in experimental animals in the early stages of drug-induced diabetes. It was noticed that in the early stages of streptozotocin-induced diabetes, lesions in protein amines of rats' renal glomeruli occures in various sequential order.*

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

Introduction. Diabetes mellitus (DM) and diabetic nephropathy as one of its serious complications, is a world leader among the causes of terminal renal failure [4, 9, 13]. Diabetes affects the renal vessels, arteries, renal tubules and glomeruli. Kidney malfunction occurs due to changes in the metabolism of lipids and carbohydrates. The disease affects 75% of people suffering from diabetes.

This kidney damage leads to disability of the patient and can significantly shorten their life. It is naturally, that the attention of many researchers is drawn to the dysfunction of the renal glomerular apparatus — epithelial, endothelial and mesangial cells in the pathogenesis of diabetic renal affection [5, 6, 11, 12].

An early disability and high mortality rate of patients with diabetes and its complications, explain a necessity of the deepest research into the mechanisms of this disease [7, 8, 10, 14], as well as an active detection and timely treatment of diabetic complications in early stages of its course [8, 15, 16].

We know that in diabetes the two independent processes such as non-enzymatic glycosylation of proteins and their oxidative modification are involved as damage mechanisms. Both processes have a reduced number of protein amines.

We used a histochemical approach, which allows to evaluate the modifications in the protein amines. The approach is based on the use of staining techniques of tissue histological sections of experimental animals with bromphenol blue, when proteins with different ratios of amino and carboxyl groups are stained differently. In particular, if amines prevail in the protein, the staining is characterized by blue and when carboxyl groups are predominant, the staining is red [3].

The color is evaluated quantitatively on digital micrographs by the RGB system and computer microspectrophotometry, particularly through the ratio R / B. These studies in diabetes at the time had not been performed yet [3].

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.

Materials and methods. The experiment was conducted on 32 male mature non-linear albino rats, weighing 0.17–0.20 kg. The animals were devided into four groups. The first (I) — control group (n = 7), was on the standard mode of feeding, lighting and housing. The experimental groups of animals (II-n = 8; III-n = 9 and IV-n = 8) were administered semiexpendable streptozotocin intraperitoneally (Sigma, USA) at a dose of 70 mg / kg [2]. In the second group of animals the slaughter and relevant research were conducted 11 days after streptozotocin administration; the performance of the animals in the third group was studied 21 days later, in the fourth one- after 31 days, respectively.

To study the basic values of the renal functions, we slaughtered the animals under light ether anesthesia, in compliance with the EEC Directive №609 (1986) and MOH of Ukraine №690 of 23.09.2009. "On the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes" The quantitative assessment of proteins in histochemical preparations stained with bromphenol blue by Mikel Calvo technique, was performed by a computer-based microspectrophotometry according to the ratio R / B. Differences between the groups of research were performed by Student's test.

Results and discussion. In histological sections of the animal renal glomeruli stained with bronphenol blue, one could see the following structures: endotheliocytes together with subendothelial basement membrane, mesangiocytes with mesangial matrix, podocytes and the basement membrane of the Bowman's capsule.

Considering the fact that diabetes is a disease that is characterized by the development of angiopathy, our attention was, first of all, drawn to the endotheliocytes and to the subendothelial basement membrane of the renal glomerular capillaries. They were visually characterized by an intense color with some predominance of red. This subjective conclusion was confirmed by means of the above mentioned quantitative study based on the computer microspectrophotometry, as the ratio R / B was always greater than one.

In endothelial cells the ratio R / B together with subendothelial basal membrane was: in intact animals — 1,09 ± 0,014, in experimental animals on the 11^{th} day of the experiment — 1,10 ± 0,018, on the 21^{st} day — 1,38 ± 0,012, on the 31^{st} day — 1,44 ± 0,017 respectively (Table 1).

The following findings were obtained for mesangiocytes with mesangial matrix: in the intact animals — 1,13 ± 0,019, in experimental animals on the 11^{th} day — 1,49 ± 0,017, on the 21^{st} day — 1,68 ± 0,016 and on the 31^{st} day — 1,79 ± 0.019 respectively (Table 2).

As a result of our research, it was noticed that the average increase in the ratio R / B in mesangial matrix and mesangiocytes, compared to intact animals, had taken place by the 11^{th} day of the experiment. This may indicate that the mesangiocyte proteins influence the processes of oxidative modification of proteins in a greater extent than those of their non-enzymatic glycosylation.

Table 1

Ratio R / B in the renal glomerular basement membrane with endotheliocytes in drug-induced diabetes at different time of the experiment (X ± sx)

Group name / **Ratio R/B**	Experimental group I (n=7)	Experimental group II (n=8)	Experimental group III (n=9)	Experimental group IV (n=8)
Ratio R/B	1,09±0,014	1,10±0,018	1,38±0,012	1,44±0,017
Difference probability(P) from the intact animals and in evolution		Pi>0,05	Pi<0,001 P11<0,001	Pi<0,001 P11<0,001 P21=0,016

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).

Table 2

Ratio R/B in the mesangial matrix and in mesangiocytes in case of drug-induced diabetes mellitus at different time of the experiment (X±sx)

Group name / **Ratio R/B**	Experimental group I (n=7)	Experimental group II (n=8)	Experimental group III (n=9)	Experimental group IV (n=8)
Ratio R/B	1,13±0,019	1,49±0,017	1,68±0,016	1,79±0,019
Difference probability(P) from the intact animals and in evolution		Pi<0,001	Pi<0,001 P11<0,001	Pi<0,001 P11<0,001 P21=0,005

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).

In contrast to the previously described structures of the renal glomerulus, podocytes were predominately colored blue. This is indicated by the fact that the ratio R / B in these cells was always below one.

Podocytes were characterized by the following mean figures of ratio R / B: in intact animals — 0,84 ± 0,012, in experimental animals on the 11th day — 0,86 ± 0,014, on the 21st day — 0,86 ± 0,018 and on the 31st day — 0.98 ± 0,018 respectively. As you can see, the changes in these structures occurred later (not earlier than on the 31st day) (Table 3).

Changes in the basement membrane of the Bowman's capsule were characterized by the following values: in intact animals — 1,11±0,012, in experimental animals on the 11th day — 1,13±0,019, on the 21st day — 1,39±0,014 and on the 31st day- 1,48±0,018 respectively (Table 4).

Table 3

Ratio R/B in podocytes in drug-induced diabetes mellitus at different time of the experiment (X±sx)

Group name / **Ratio R/B**	Experimental group I (n=7)	Experimental group II (n=8)	Experimental group III (n=9)	Experimental group IV (n=8)
Ratio R/B	0,84±0,012	0,86±0,014	0,86±0,018	0,98±0,018
Difference probability(P) from the intact animals and in evolution		Pi>0,05	Pi>0,05 P11>0,05	Pi=0,001 P11=0,003 P21=0,004

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).

Table 4

Ratio R/B in the basal membrane of the Bowman's capsule in drug-induced diabetes mellitus at different time of the experiment (X±sx)

Group name / **Ratio R/B**	Experimental group I (n=7)	Experimental group II (n=8)	Experimental group III (n=9)	Experimental group IV (n=8)
Ratio R/B	1,11±0,012	1,13±0,019	1,39±0,014	1,48±0,018
Difference probability(P) from the intact animals and in evolution		Pi>0,05	Pi<0,001 P11<0,001	Pi<0,001 P11<0,001 P21=0,007

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).

It should be noted that the ratio of R / B in the basement membrane of the Bowman's capsule had the same dynamics as the endotheliocytes and subendothelial membrane of the renal glomerular capillaries.

Conclusions. In streptozotocin-induced diabetes, an affection of amino groups of proteins in the renal glomeruli occurs in mesangiocytes with mesangial matrix first- on about the 11th day; the next to be affected are endotheliocytes with subendothelial basal membrane as well as those in the basal membrane of the Bowman's capsule- on the 21st day of our study and podocytes were the last- on the 31st day.

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.

References:

1. Gavaleshko V. P. Histological changes in kidneys at diabetes mellitus, complicated by partial global ischemia-reperfusion / V. P. Gavaleshko // Clinical Anatomy and Operative Surgery — 2012. — V.11, №3. — P. 62–65.

2. Galenova T. I. The modeling of experimental streptozotocin-induced II type diabetes in rats / T. I. Galenova, V. V. Konopelniuk, O. M. Savchuk, L. I. Ostapchenko // Physics of Alive. — 2010. — V.18, №3. — P. 50–54.

3. Davydenko I. S Histochemical peculiarities of oxidative modification of proteins in glomeruli cells at acute postinfectional glomerulonephritis / I. S. Davydenko, O. M. Davydenko // Bukovinian Medical Journal. — 2012. — V. 16, №3 (63). — Part 2. — P. 106–108.

4. Loboda O. M. Mechanism of development and progression of diabetic nephropathy / O. M. Loboda, I. O. Dudar, V. V. Alekseeva // Clinical Immunology. Allergology. Infectology. — 2010. — №9–10 (38–39). — P. 46–50.

5. Maidannyk V. G. Molecular mechanisms of kidneys' damage at diabetes mellitus in children (review article) / V. G. Maidannyk, Ye.A. Burlaka // Pediatrics, Obstetrics and Gynecology. — 2010. — №3. — P. 34–47.

6. Rebrov B. A. Kidneys' damage at diabetes mellitus / B. A. Rebrov // International Endocrinology Journal. — 2011. — № 2(34). — P. 51–55.

7. Scrobonska N. A. Diabetic nephropathy: some untraditional factors of pathogenesis, main ways of diagnostics and treatment (review article and personal results) / N. A. Scrobonska, T. S. Tcymbal // Family Medicine. — 2011. — №4. — P. 18–22.

8. Bodnar I. A. Role of glomeruli cells dysfunction in the development of diabetic nephropathy / I. A. Bodnar, V. V. Klymontov // Problems of Endocrinology. — 2006. — V. 52, №4. — P. 45–49.

9. Hutorska L. A. Prevalence, absolute and relative risk of the development of diabetic nephropathy in patients with diabetes mellitus / L. A. Hutorska // Bukovinian Medical Journal. — 2012. — V.16, №4(64). — P. 170–174.

10. Shularenko L. V. Chronical diabetic renal disease: modern view on the problem / L. V. Shularenko // Endocrinology. — 2013. — Vol. 18, No 1. — P. 73–82.

11. The Attenuation of Moutan Cortex on Oxidative Stress for Renal Injury in AGEs-Induced Mesangial Cell Dysfunction and Streptozotocin-Induced Diabetic Nephropathy Rats / Mingua Zhang, Liang Feng, JunfeiGu [et al.] // Oxidative Medicine and Cellular Longevity. — 2014. — Vol. 18.– P. 1–13.

12. Dranovalli S. The Pathogenesis of Diabetic Nephropathy / S. Dranovalli, I. Duka, G. L. Bakris // Nat. Clin. Pract. Endocrinol. Metab. — 2008. — N.2. — P. 444–452.

13. Evans T. C. Diabetic Nephropathy / T. C. Evans, Capell P. // Clinical Diabetes. — 2000. — N. 1. — P. 198–214.

14. Forst T. Role of C-Peptide in the Regulation of Microvascular Blood Flow / T. Forst, T. Kunt, B. Wilhelm // Exp. Diabetes Res. — 2008. — N.6. — P. 176–245.

15. Hills C. E. Cellular and Physiological Effects of C-Peptide / C. E. Hills, N. J. Brunskill // Clin. Sci (Lond). — 2009. — Vol.116 (7). — P. 565–574.

16. Palmer J. P. C-Peptide in the Natural History of Type 1 Diabetes /J. P. Palmer // Diabetes Metab. Res. Rev. — 2009. — Vol.25 (4). — P. 325–328.