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THE EFFECT OF NADP ON SOME VALUES OF THE RENAL SECRETORY FUNCTION IN RATS WITH STREPTOZOTOCIN-INDUCED DIABETES MELLITUS

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Diabetes has been a significant social problem, since it leads both to disability and mortality. The experiment involved 32 sexually mature nonlinear males of white rats. Experimental groups of animals were administered a single streptozotocin dose of 70 mg/kg intraperitoneally and NADP — 30 mg/kg. The animals in the experimental groups were slaughtered and studied on the 11th, 21st and 31st days after streptozotocin administration. The studies have shown that, along with the development of proteinuria, kidney damage is manifested by the primary increase in glomerular filtration rate and changes in diuresis in experimental animals.

Conclusion. The primary possible mechanisms of the development of diabetic nephropathy are the disorders in glycosylation processes, overload of functioning nephrons with protein, and increasing energy expenditure in the proximal and distal parts of the nephron.

Key words*: diabetes mellitus, nephropathy, hyperfiltration, streptozotocin, diuresis.*

Introduction

At present, about 382 million people with diabetes mellitus (DM) live in the world. According to domestic and foreign scientists, without unified and coordinated measures to overcome the epidemic of this disease, their number will reach 592 million by 2035. According to Lyubov Sokolova — Head of the Department of Diabetes of the State Institution "V. P. Komisarenko Institute of Endocrinology and Metabolism of the NAMS of Ukraine" DM stands third in Ukraine after cardiovascular and oncological diseases [1].

Despite the large number of papers dealing with the study of types 1 and 2 diabetes mellitus (DM), the etiology and pathogenesis of its development have not been definitely determined yet. The severity of this disease is due, above all, to the gravity and progress of its complications. The incidence of certain complications of DM depends on the compensation of the disease and its duration. But some complications of diabetes can be diagnosed both at the beginning of this disease and during its long course [5, 8]. Among the typical complications of diabetes mellitus is vascular damage, which manifests itself through a variety of micro- and macroangiopathies. Among the prognostically unfavorable complications of diabetes is diabetic nephropathy (DN). DN leads to proteinuria and, afterwards, to a reduced glomerular filtration rate (GFR) with a gradual development of chronic kidney disease [7, 9].

The incidence of DN varies from 6 to 40 % or more, depending on the duration of the disease, the adequacy of treatment, and the degree of compensation for this ailment [10, 11].

The leading factors in the pathogenesis of DN development are metabolic and hemodynamic disorders. In the case of DN all cellular elements of the kidney are affected: glomerular endothelium, mesangial cells, podocytes and epithelium of the renal tubules. The main provocative factor in diabetes is hyperglycemia, which causes a

disruption of metabolic processes and leads to accumulation of components of the extracellular matrix and subsequent fibrosis.

The degree of damage to the tubules depends on such factors as high levels of glucose and the content of glycosylation end products, as well as a large amount of protein that is filtered through the glomerular capillary barrier [7, 10].

To study the pathophysiological mechanisms of the onset and progression of DN, scientists use appropriate experimental models that accurately reproduce the natural course of this vascular complication of diabetes in people. Streptozotocin-induced diabetes mellitus (STZ) is commonly used among non-genetic models of diabetes [3, 4]. It is an antibiotic with oncostatic action that selectively penetrates into pancreatic beta cells using a GLUT-2 carrier and causes a shortage of NAD + cofactor reserve and later of energy substrates as ATP, which inevitably leads to beta-cell necrosis. This process is enhanced by the activation of free radical oxidation associated with the generation of peroxynitrite from the excess of nitric oxide produced by the nitroso group of STZ. [3, 4].

Objective

To identify the features of disorders in the values of the excretory renal function in the early stages of the development of experimental streptozotocin-induced diabetes mellitus while administering NADP.

Materials and methods

The study involved 32 sexually mature nonlinear males of white rats, weighing from 0.17 to 0.20 kg. The animals were divided into four groups. The first group (I) was a control one (n = 9), whose animals were on the standard feeding, lighting and maintenance. The experimental groups of animals (II–n = 8; III–n = 8 and IV–n = 7) were administered streptozotocin (Sigma, USA) at a single dose of 70 mg / kg intraperitoneally [2,6]. The animals of the 2nd group were slaughtered and studied 11 days after the administration of streptotsotocin, the values in the animals of the 3rd group were examined after 21 days, IV — after 31 days, respectively. The experiment involved the animals whose glycemic level exceeded 10 mmol/l. The rats of the experimental groups were administered a NADP solution intraperitoneally at a dose of 30 mg/kg of body weight on isotonic sodium chloride solution.

In order to investigate the necessary values, the slaughter of animals was carried out under a light etheric anesthesia, following the provisions of the EU Directive No. 609 (1986) and the Order of the Ministry of Health of Ukraine No. 690 of September 23, 2009 "On Measures for the Further Improvement of Organizational Norms of Work with the Use of Experimental Animals". To evaluate the function of the vascular-glomerular apparatus of the kidney, the animals were loaded with water with 5 % of the body weight, and the urine was collected for 2 hours. The probability of difference of values was determined using the Student t-criteria. In the tables, the values of probability ("p") are given only for probable (p = 0.05 or less) differences of the studied values.

Results and discussion

As one can see from the table data, NADP administration does not significantly affect the excretory renal function values. For instance, the diuresis of experimental animals on the 11th day of the experiment was 38.3 % lower than that of the control group of animals. Moreover, there were no significant differences between the values in the intact rats and those in the experimental groups on the 21st and 31st days of the experiment. Changes in diuresis may be associated with disorders in both glomerular filtration and tubular reabsorption.

While studying the state of these processes, we determined that on the 11th day the glomerular filtration does not decrease, as it is described in many clinical studies on diabetes, but, on the contrary, significantly increases by more than twice. Moreover, on the 31st day of the experiment this

value exceeds the control ones by 228,4 %. An increase in GFR indicates the effective excretion of nitrogen metabolites from the body (in this case it is creatinine). Concentration of creatinine in the urine is increasing over the entire period of observation.

It should be noted that the decrease in diuresis on the 11th day of the experiment can be explained by the fact that in the extracellular fluid osmolality increases, as a result of an increase in glucose concentration, simultaneously with an increase in the content of sodium. Hyperosmolarity stimulates the increase of vasopressin secretion, which, due to the growth of water reabsorption in the collecting tubes, reduces water diuresis. The subsequent restoration of the level of diuresis in animals from the experimental groups can be related to the effect of the NADP.

The growth of glomerular filtration rate leads to an increase in the loss of protein with the urine. In general, proteinuria is known to indicate kidney involvement in the pathological process (increased filtration and reduced reabsorption), but in these studies, increased protein excretion was found during the observation period. Moreover, while on the 11th day of the experiment the increase in protein excretion was only recorded when calculated for 2 hours of diuresis, on the 21st and the 31st day of the year this value increased reliably both in the calculation for 2 hours and with its standardization for the GF (table). In general, protein excretion on the 11th day of the experiment exceeds by 31.3 % the findings in the intact animals, it increases by 1.8 times on the 21st day ($p < 0.001$) compared with the control ones, and by 2, 8 times on the 31st day ($p < 0.001$).

It can not be excluded that proteinuria is indeed one of the signs of renal dysfunction, but the leading mechanism is probably the further overload of the nephron with filtered protein, which, with restriction of reabsorption in the proximal tubule, leads to its loss of urine.

Conclusion

The obtained results give grounds to assume that the administration of NADP does not lead to either an obvious or a hidden reduction of the number of functionally active renal glomeruli in the initial period of diabetes development. Administration of NADP to the animals with experimental DM only partially improves the values of the excretory renal function. Probable disorders in the processes of glycosylation, overload of functioning nephrons with protein and increasing energy expenditure in the proximal and distal parts of the nephron lead to kidney damage and subsequent development of diabetic nephropathy.

Prospects of further research

Taking into account these findings,

Table

Changes in some values of the renal excretory function in rats with streptozotocin-induced diabetes mellitus and NADP administration

Value	Control, $n = 9$	Streptozotocin-induced DM+NADP		
		11th day, $n = 8$	**21st day**, $n = 8$	**31st day**, $n = 7$
Diuresis, ml/2 h	4,23 ± 0,21	2,61 ± 0,21 $p < 0,01$	4,59 ± 0,42	4,34 ± 0,39
Concentration of creatinine in urine, mmol/l	0,68 ± 0,03	1,86 ± 0,37 $p < 0,01$	1,43 ± 0,27 $p < 0,01$	1,08 ± 0,13 $p < 0,05$
Concentration Index of Endogenous Creatinine, St.U.	22,12 ± 0,65	38,41 ± 9,55 $p < 0,05$	48,41 ± 10,58 $p < 0,01$	24,74 ± 5,49
Glomerular Filtration Rate, µl/min	412,82 ± 26,78	810,84 ± 93,33 $p < 0,001$	819,83 ± 50,25 $p < 0,001$	942,73 ± 284,16 $p < 0,001$
Extract of protein, mg/2 h	0,118 ± 0,014	0,155 ± 0,021 $p < 0,05$	0,223 ± 0,101 $p < 0,001$	0,334 ± 0,035 $p < 0,001$
Extract of protein, mg/100 µl of GF	0,029 ± 0,003	0,026 ± 0,006	0,046 ± 0,006 $p < 0,05$	0,052 ± 0,052 $p < 0,001$

Note. n — number of animals in the group; p — probability of difference between the experimental and the control groups of animals, GF — glomerular filtrate.

further elucidation of renal function changes with the administration of NADP in the early stages of the experimental DM development will be relevant in order to find the ways to prevent DN.

References/Литература

1. Tkachenko VI Analysis of prevalence and incidence of diabetes mellitus among the world population and Ukraine for 2003-2013. Drugs of Ukraine. 2014; 4 (21): 55-59. / Ткаченко ВІ. Аналіз поширеності та захворюваності на цукровий діабет серед населення світу та України за 2003-2013 рр. Ліки України. 2014; 4 (21): 55-59.
2. Bequer L, Gymez T, Molina JL. Streptozotocin diabetogenic action in an experimental neonatal induction model. Biomedica. 2016; 36 (2): 230-8.
3. Bruna BN, Luis HC. The role of progranulin in diabetes and kidney disease. Diabetol. Metab. Syndr. 2015; 7: 117-25.
4. Boonloh K, Lee ES, Kim, Hong M, Kwon MH, Kim YM, Pannangpetch P. Rice bran protein hydrolysates attenuate diabetic nephropathy in diabetic animal model. European journal of nutrition. 2016; 1-12. DOI: 10.1007/s00394-016-1366-y.
5. El Din UAAS, Salem MM, Abdulazim DO. Diabetic nephropathy: Time to withhold development and progression A review. Journal of advanced research. 2017; 8 (4): 363-73. doi: 10.1016/j.jare.2017.04.004.
6. Herrera GA, Turbat-Herrera EA, Teng J. Animal Models of Light Chain Deposition Disease Provide a Better Understanding of Nodular Glomerulosclerosis. Nephron. 2016; 132 (2): 119-36.
7. Liu X, Mo Y, Gong J. Puerarin ameliorates cognitive deficits in streptozotocin-induced diabetic rats. Metab. Brain Dis. 2016; 31 (2): 417-23.
8. Mohammad S, Basel M, Abozor L, Khalid H, Zawawi G. The relationship between periapical lesions and the serum levels of glycosylated hemoglobin and C-reactive protein in type 2 diabetic patients. Saudi Med. J. 2017; 1 (38): 36-40.
9. Mora-Fernбndez C, Domнnguez-Pimentel V, de Fuentes MM, Gyrriz JL, Martнnez-Castelao A, Navarro-Gonzбlez JF. Diabetic kidney disease: from physiology to therapeutics. J Physiol. 2014; 592 (18): 3997-4012. doi: 10.1113/jphysiol.2014.272328.
10. Romero MJ, Lucas R, Dou H. Role of growth hormone-releasing hormone in dyslipidemia associated with experimental type 1 diabetes. Proc. Natl. Acad Sci USA 2016; 113 (7): 1895-1900.
11. Sen Zh, Dongjie W, Nina X. Nicousamide protects kidney podocyte by inhibiting the TGFв receptor II phosphorylation and AGE-RAGE signaling. Am. J. Transl. Res. 2017; 9 (1): 115-25.

Резюме

ВПЛИВ НАДФ НА ОКРЕМІ ПОКАЗНИКИ ЕКСКРЕТОРНОЇ ФУНКЦІЇ НИРОК ПРИ СТРЕПТОЗОТОЦИН-ІНДУКОВАНОМУ ЦУКРОВОМУ ДІАБЕТІ У ЩУРІВ

Грицюк М.І.

Цукровий діабет залишається значною соціальною проблемою, оскільки веде не лише до втрати працездатності, а й до інвалідизації та летальних наслідків. Експеримент проведено на 32 статевозрілих нелінійних самцях білих щурів. Дослідним групам тварин одноразово внутрішньоочеревинно уводили стрептозотоцин у дозі 70 мг/кг, НАДФ — 30 мг/кг. У дослідних групах тварин забій та відповідні дослідження проводили через 11 діб після уведення стрептозотоцину, через 21 та 31 добу відповідно. Проведені дослідження показали, що поряд із розвитком протеїнурії ураження нирок проявляється первинним зростанням швидкості клубочкової фільтрації та змінами діурезу дослідних тварин.

Висновок. Первинними можливими механізмами розвитку діабетичної нефропатії є порушення процесів глікозилювання, перевантаження функціонуючих нефронів білком і зростаючі енерговитрати в проксимальному і дистальному відділах нефрону.

Ключові слова: *цукровий діабет, нефропатія, гіперфільтрація, стрептозотоцин, діурез.*

Резюме

ВЛИЯНИЕ НАДФ НА ОТДЕЛЬНЫЕ ПОКАЗАТЕЛИ ЭКСКРЕТОРНОЙ ФУНКЦИИ ПОЧЕК ПРИ СТРЕПТОЗОТОЦИН-ИНДУЦИРОВАННОМ САХАРНОМ ДИАБЕТЕ У КРЫС

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Сахарный диабет остается значительной социальной проблемой, поскольку ведет не только к потере работоспособности, но и к инвалидизации и летальным исходам. Эксперимент проведен на 32 половозрелых нелинейных самцах белых крыс. Экспериментальным группам животных однократно внутрибрюшинно вводили стрептозотоцин в дозе 70 мг/кг, НАДФ — 30 мг/кг. В опытных группах убой животных и соответствующие исследования проводили через 11 суток после введения стрептозотоцина, через 21 и 31 сутки соответственно. Проведенные исследования показали, что, наряду с развитием протеинурии, поражения почек проявляются первичным ростом скорости клубочковой фильтрации и изменениями диуреза опытных животных.

Вывод. Первичными возможными механизмами развития диабетической нефропатии является нарушение процессов гликозилирования, перегрузки функционирующих нефронов белком и растущие энергозатраты в проксимальном и дистальном отделах нефрона.

Ключевые слова: *сахарный диабет, нефропатия, гиперфильтрация, стрептозотоцин, диурез.*

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РОЛЬ ОСТЕОПРОТЕГЕРИНУ В МЕХАНІЗМАХ РОЗВИТКУ ВТОРИННОГО ОСТЕОПОРОЗУ ПРИ МОДЕЛЮВАННІ ПАРОДОНТИТУ

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В молекулярних механізмах формування остеопорозу ключову роль відіграє остеопротегерин (ОПГ), сироватковий рівень якого зростає із прогресуванням захворювання. Був досліджений взаємозв'язок між рівнем остеопротегерину й антагоніста рецептора інтерлейкіну-1 у механізмах розвитку вторинного остеопорозу при моделюванні пародонтиту. Виявлено збільшення вмісту ОПГ у сироватці крові тварин у групі із пародонтитом, що можна розглядати як компенсаторну реакцію на підвищення активності остеокластів, а також як Т-клітинну імунну відповідь на запалення. Кореляції рівнів цитокінів у тварин інтактної та експериментальної груп свідчать про те, що є взаємозв'язок у системі регуляції ремоделювання кісткової тканини.

Характер зміни рівнів ІЛ-1РА і остеопротегерину має зворотню спрямованість процесів, що дозволяє припустити існування механізму зворотнього негативного зв'язку між порушеннями кісткового метаболізму й запальним процесом в пародонті, що реалізується у вигляді міжклітинних медіаторів.

Остеопротегерин відіграє значну роль не тільки у процесах ремоделювання кістки, але й у розвитку запального процесу в пародонті.

Ключові слова: *остеопротегерин, остеопороз*