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OBSAH

Ekonomika

| | |
|--|----|
| Ignatieva Iryna, Khomenko Oleksandr. Transformace potenciálu sociálně ekonomických změn pod vlivem formace způsobilosti personálu k cizím jazykům | 9 |
| Gnatenko Olena, Lys Nadiia. Faktory vlivu na konkurenceschopnost ukrajinských pojišťoven | 17 |
| Savchuk Natalia, Sienin Oleksandr. Základní rozporы globálního pojmu finanční stability | 23 |
| Kriuchkova Nataliya, Sechnyak Alexey. Fiskální politika: teoretické definice, struktura a klasifikace v ukrajinském vědeckém pojednání | 30 |
| Slezko Tatyana. Některé aspekty, které definují účetnictví jako instituce socioekonomického systému | 39 |
| Lentner Csaba, Zéman Zoltán. Manipulace s krizi - role v ekonomice..... | 45 |

Filozofie a teologie

| | |
|--|----|
| Dudareva Olga. Počátky mimetické krize literární teorie: lingvistický aspekt..... | 59 |
| Turenko Vitalii. Zda milostné příběhy patří k dějinám: pro et contra | 65 |
| Filonenko Boris. Barva a reverzní intencionalita. Fenomenologické chromatiky po E.Husserlu | 73 |
| Christokin Gennadii. Metodologie teologie řecké patristiky | 79 |
| Škil' Svetlana. Reakce ukrajinské náboženské komunity na "Havanské prohlášení" papeže Františka a patriarchy Kirilla..... | 85 |

Lékařství a fyziologie

| | |
|---|-----|
| Braun Yuliya, Beloklická Galina, Grigorovský Valeriy. Zkoumání korelačních závislostí morfologických ukazatelů stavu tkáně parodontu u pacientů s generalizovanou paradentózou v průběhu chirurgické fáze komplexní léčby | 91 |
| Grytsiuk Maryana. Porušení určitých struktur glomerul ledvin krys v drogami způsobeném diabetes mellitus za použití histochemické techniky | 100 |
| Guliuk Anatoliy, Kogan Lubov. Změny biochemických indikátorů orální tekutiny při léčbě chronického katarálního zánětu dásní u dětí, dříve prooperováných pro kombinovaný rozštěp měkkého a tvrdého patra | 105 |
| Žuk Dmitriy, Počtar Viktoriya, Šnaider Stanislav. Laserová terapie ve fázi epitelizace erozí, vředů sliznice dutiny ústní při aphthosis Setton | 112 |
| Kartel Nikolay, Ivanov Leonid, Nardyd Oleg, Cherkashyna Yana, Okotrub Alexander, Derymedved Lyudmyla, Vereitinova Valentina, Tarasenko Olga. Vyhodnocení vlivu uhlíkových nanotrubiček na mitochondriální aktivity buněk v tkáních různých orgánů prostřednictvím metody otačení sondy | 121 |
| Usenko Aleksandr, Savická Irina, Kosenko Dariya, Novická Irina. Histomorphologické změny v submandibulárních slinných žlázách po resekci jater (experimentální zkoumání) | 132 |
| Litvinenko Nataliya, Varická Anna, Denisov Aleksey. Účinnost krátkých režimů chemoterapie s intravenózním použitím anti-TB léčiv u pacientů s multirezistentní tuberkulózou | 142 |
| Polischuk Serhey. Klinický pohyb hojení zhmožděných tržních ran obličeje na pozadí dysfunkce hepatobiliárního traktu | 150 |
| Savčuk Oleg. Partnerství veřejné a soukromé ve stomatologii | 159 |
| Saveliyeva Nataliya. Klinický průběh chronické zobecněné paradentózy u pacientů s enterobiózou | 165 |

Biologie

- Šikula Rostislav.** Organizace nezávislé vědecké a pedagogické práce s budoucími učiteli odvětví přírodopisu s využitím prostředků muzejní pedagogiky 173

Geologie

- Sergey Fedoseyenko.** Metody dálkového profilového půdního průzkumu pro určení typů sedimentů/spodních usazenin 179

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 occurs 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 divided 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 bromphenol blue, one could see the following structures: endotheliocytes together with subendothelial basement membrane, mesangiocyes 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 — $1,09 \pm 0,014$, in experimental animals on the 11th day of the experiment — $1,10 \pm 0,018$, on the 21st day — $1,38 \pm 0,012$, on the 31st day — $1,44 \pm 0,017$ respectively (Table 1).

The following findings were obtained for mesangiocyes with mesangial matrix: in the intact animals — $1,13 \pm 0,019$, in experimental animals on the 11th day — $1,49 \pm 0,017$, on the 21st day — $1,68 \pm 0,016$ and on the 31st day — $1,79 \pm 0,019$ 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 mesangiocyes, compared to intact animals, had taken place by the 11th day of the experiment. This may indicate that the mesangiocye 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 ($X \pm sx$)

| Group name | 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 mesangiocyes in case of drug-induced diabetes mellitus at different time of the experiment ($X \pm sx$)

| Group name | 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 mesangioctyes 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.

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