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Clinical and laboratory assessment of hormonal and metabolic disorders in experimental animals with alloxan, streptozotocin and dithizone diabetes

The basic place in experimental diabetology is engaged by chemical models of diabetes. In these patterns, β-cells in pancreatic islet tissue are selectively targeted by diverse agents. Pathogenetic mechanisms of hormonal and metabolic disorders in animals were studied on models of alloxan, streptozotocin and dithizone diabetes. The experiment was carried out on male Wistar rats. Three models of experimental diabetes were created. The groups were equal in terms of observation time (6, 12, 24 months) and number. A complex of laboratory-biochemical methods of lipid and protein metabolism was consumed. Serum enzymes were also determined. The level of glycemia in the studied animals after the introduction of alloxan and streptozotocin ranged from 13.4 to 18.6 mmol/l, glucosuria, polydipsia, polyphagia were observed. A significant ($p<0.01$) increase in glycemia by 3.10 times, glycated hemoglobin - by 3.16 times, a decrease ($p<0.01$) of IRI - by 3.76 times and C-peptide - by 2.05 times in animals with streptozotocin diabetes compared with intact animals. Serious lipid metabolism disorders were discovered in alloxan, streptozotocin, and especially dithizone diabetes (24 months of follow-up). This was manifested in a significant ($p<0.01$) increase in the level of triglycerides - by 1.66 times, atherogenic index ($p<0.01$) - by 4.43 times and a decrease ($p<0.05$) in the level of HDL - by 1 43 times compared with the intact CI3 group, as well as an increase in FFA, TCH, LDL, VLDL. In animals with alloxan and dithizone diabetes, the functional activity of the pituitary gland was lessened (the level of growth hormone and CTH ($p<0.01$), the adrenal cortex and pancreatic α-cells ($p<0.01$), the activity of the sympathoadrenal system was enlarged, which was reaffirmed by a significant ($p<0.01$) by an increase in the level of adrenaline, noradrenaline - by 1.42 times. An above pronounced suppression of the endocrine glands was observed in animals with streptozotocin diabetes.

Keywords: models of experimental alloxan, streptozotocin and dithizone diabetes; glycated hemoglobin, C-peptide, indicators of lipid and protein metabolism, indicators of corticotropic hormone, adrenaline, norepinephrine, cortisol, free hydrocortisone; glucagon, insulin.

Introduction

Research in recent decades has shown a marked rise in the incidence of diabetes mellitus. 80-90% are patients with diabetes mellitus type 2 [1-3], and therefore, this medical problem is of excellent social and economic importance, and also increases the workload of general practitioners in the Republic of Kazakhstan [4].

However, there is still no consensus on which of the listed factors (insulin resistance or defective β-cell function) is primary. Hyperglycemia itself can be the reason of both insulin resistance and β-cell failure. Studies [5, 6] assert the multifactorial etiology of type 2 diabetes mellitus and the considerable role of non-genetic (external) factors in the development of diabetes, for example, eating disorders [7]. According to the literature data [8], the main place in experimental diabetology was occupied by chemical models of diabetes, in which β-cells of the islet tissue of the pancreas were selectively affected by various agents. The advantage

of these models lays in the preservation of the excretory function of the pancreas and in the absence of surgical traumatization of animals [9-11].

Alloxan and streptozotocin diabetes are the most widely used chemical models of experimental diabetes mellitus. Their benefit lies in the relative ease of reproduction, high selectivity of effects on β-cells, low toxicity of the diabetogenic doses of drugs used, the possibility of obtaining diabetes of varying severity and continuation in almost all animal species, including small laboratory animals. This makes it probable to manage research on a high amount of material [12-14].

The pathogenesis of dithizone diabetes was studied in some detail. Dithizone, which is chemically diphenylthiocarbazone, combines with zinc in the pancreatic islets 2-5 minutes after administration. The resulting zinc dithizonate breaks β-cells, causing insufficiency of the insular apparatus. Dithizone in diabetogenic doses does not have a direct toxic effect on the body of rabbits. Parenchymal dystrophy of the liver, kidneys, lungs and heart, as well as the initial signs of fibrosis of these organs, were found in the chronic course of the disease, as a result of diabetic and toxic effects of heavy metal salts, defiant to metabolic disturbance [15-24].

Of specific interest is the pathology of the endocrine organs. Inspection of the adenohypophysis in alloxan diabetes revealed dystrophy with degranulation of the cytoplasm of basophils, necrosis of individual cells, and further development of hyalinosis and cystic degeneration. The weight of the pituitary gland decreased. The content of growth hormone in it did not vary with newly developed diabetes, and decreased with chronic diabetes. Sharp hyperemia and dystrophic changes in cells were noted in the adrenal glands in acute forms of alloxan diabetes. A magnification in the total weight of the adrenal glands with a sharp expansion of the fascicular zone and narrowing of the glomerular zone was revealed in chronic diabetes lasting more than a year. Foci of destruction were often discovered in the fascicular and reticular zones, necrosis of individual cells – in the cerebral. These changes were accompanied by an increase in the production of steroids and a decrease in the content of ascorbic acid [25, 26]. The expressiveness of the described changes, as well as a decrease in the content of adrenaline in organs and tissues, were directly dependent on the severity of insular insufficiency [27, 28].

The goal: to carry out a comparative analysis of hormonal and metabolic disorders in animals on models of alloxan, streptozotocin and dithizone diabetes, depending on the observation period.

Experimental

Three models of experimental diabetes were constructed on 450 male Wistar rats in a chronic experiment – alloxan A (35 mg/kg), streptozotocin S (30 mg/kg) and dithizone D (32 mg/kg), identical in terms of observation periods (6, 12, 24 months) and number (50 individuals in each subgroup). Animals were shared into subgroups A1 (6 months), A2 (12 months), A3 (24 months); S1 (6 months), S2 (12 months), S3 (24 months); D1 (6 months), D2 (12 months), D3 (24 months). Intact animals of the same age groups served as control: 30 individuals in each subgroup – CI1 (6 months), CI2 (12 months), and CI3 (24 months). Maintenance, nutrition, skin care animals during the experiment and after breeding. They were performed in accordance with international requirements for the protection of animals used for scientific purposes [29, 30]. The design of this study was accepted by the Ethical Commission at the Karaganda Medical University (protocol No. 3 of 07/11/2022).

The degree of compensation of carbohydrate metabolism was predetermined by the level of glycated hemoglobin (Hb_{A1c}) in the blood, using a laboratory analyzer DCA-2000 MT (BAYER, Germany). Blood was taken from the tail vein to determine metabolic parameters in experimental animals. The concentration of C-peptide in the blood serum was defined by the method of immunoluminometric analysis "Immunotech" (Czech Republic). A set of laboratory and biochemical methods of lipid and protein metabolism by enzymatic colorimetric methods (CHOD-PAP, GPO-PAP) was carried out on an automatic biochemical analyzer "TARGA-2000" ("BIOTECHNICA INSTRUMENT", Italy).

The following blood serum enzymes were measured to assess the functional state of internal organs (according to Reitman and Frenkel): aspartate aminotransferase (ACaT), alanine aminotransferase (AlAT), lactate dehydrogenase (LDH) [31, 32]. Indicators of hormone metabolism were determined by ELISA using kits from DSL (USA) with subsequent measurement of optical density on a Spectra Classic reader from Tecan (Austria). The content of the following hormones was determined: corticotropic hormone (CTH), epinephrine, norepinephrine, cortisol, free hydrocortisone; 17-ketosteroids, 17-hydroxycorticosteroids, glucagon, insulin, growth hormone (GH); thyroid-stimulating hormone (TSH); thyroxine (T4); thyroxine (T3).

The received data were processed by the method of variation statistics. The arithmetic mean sample (M), the standard deviation (δ), and the error of the arithmetic mean (m) were determined. Significance of differences was assessed by Student's t-test. The relationship of quantitative characteristics was studied by the method of correlation analysis. The significance of the linear correlation coefficient (Pearson) and rank (Spearman) was checked on the basis of Student's t-test. The correlation coefficient r, which is calculated in Excel using the f_x function, statistical functions, the CORREL function were used to quantify the closeness of the connection [33, 34].

Results

The glycemia level of experimental animals after the injection of alloxan and streptozotocin ranged from 13.4 to 18.6 mmol/L. In addition, glucosuria, polydipsia, polyphagia were observed in rats, the weight of rats decreased by 30-60 g, and then increased to 180–220 g. The state of carbohydrate balance is introduced in Table 1. In animals of all three models, the level of glycemia tended to rise depending on the continuance of observation, so the level of glucose was higher at 24 months of supervision. Thus, a significant increase in the level of glucose in the blood ($p<0.01$) by 2.72 times was revealed in animals of the A3 group compared with CI3. The glycemia index was higher in groups with streptozotocin diabetes than in animals with alloxan and dithizone diabetes. Thus, in animals of the S3 group ($p<0.01$), the glycemia index was in 3.10 times higher compared to the CI3 group. Glycemic indices in subgroups D1, D2 and D3 were lower than in animals with alloxan and streptozotocin diabetes, but significantly high ($p<0.05$) by 2.38 times compared with CI3 group.

Immunoreactive insulin (IRI) in intact animals was in the diapason from 4.48 to 4.52 μU/ml, while a significant reduction in IRI indicators was revealed ($p<0.01$) in animals with alloxan diabetes: in A1 group – in 1.91 times compared with CI1 group, in A2 – in 2.18 times compared with CI2 group, in A3 – in 2.28 times compared with CI3 group. The most significantly ($p<0.01$) IRI values were in animals with streptozotocin diabetes, especially in the S3 subgroup – in 3.76 times compared to CI3. In subgroups with dithizone diabetes, IRI values were reduced by 6.3% compared with control groups and ranged from 4.28 to 4.25 μU/ml (Fig. 1).

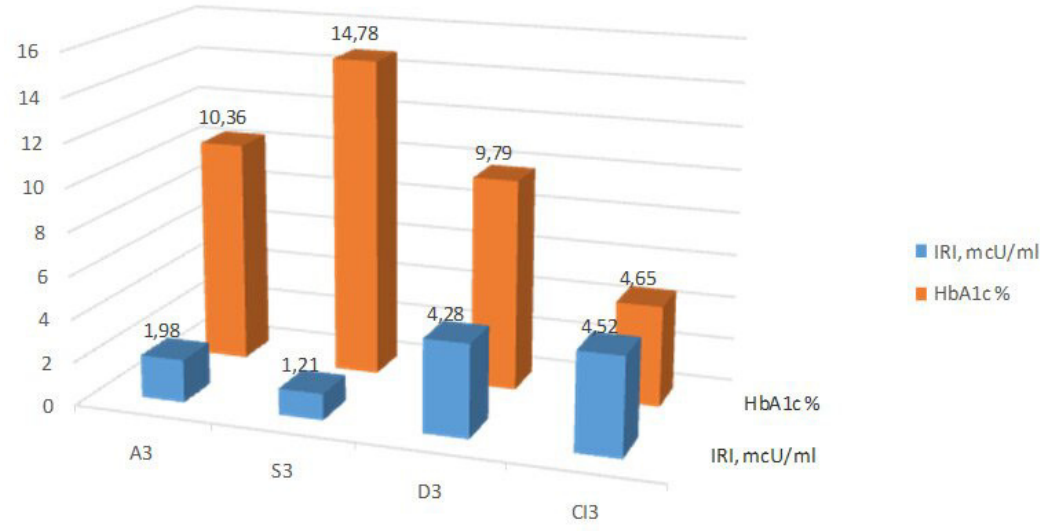

Figure 1. Indicators of IRI and Hb_{A1c} in the blood of rats with experimental diabetes (24 months of observation)

Significantly ($p<0.01$), the highest levels of glycated hemoglobin were in subgroups S1 and S3 – in 2.58 and 3.16 times higher compared to CI2 and CI3. This indicator was significantly ($p<0.01$) in 2.22 times higher in groups A1, A2 and A3 than in intact animals. HbA1c was significantly in 2.10 times higher in subgroups with dithizone diabetes than in groups CI ($p<0.01$) (Table 1).

The level of C-peptide differed in animals of group S3 with streptozotocin diabetes – it was in 2.05 time lower in comparison to group CI3. This indicator was in 1.38 time lower in animals of group A3, while the level of C-peptide was in 1.02 time higher in subgroup D3 compared to group CI3. A slight increase in the content of lactate in the blood of animals was revealed in all models of diabetes by 0.09 mmol/l compared

with intact animals. The level of pyruvates was increased in experimental animals by 1.16 times. The Lactate/Pyruvic acid ratio was lower in animals with streptozotocin diabetes, indicating progression of carbohydrate metabolism (Fig. 1).

An increase in all parameters of lipid metabolism in rats with various models of diabetes was revealed with enlarge in the observation period (24 months) in groups A3, S3, D3, and CI3 (Table 2). A rise increase in the level of FFA by 1.38 time was registered in group S3 compared to group CI3. The level of FFA in group D3 was increased by 1.23 time; in group A3 – by 1.26 time compared with the intact CI3 group.

Table 1

Indicators of carbohydrate metabolism in the blood of rats with experimental diabetes

Index	Alloxan diabetes			Streptozotocin diabetes			Dithizone diabetes			Control		
	A1 (n=50)	A2 (n=50)	A3 (n=50)	S1 (n=50)	S2 (n=50)	S3 (n=50)	D1 (n=50)	D2 (n=50)	D3 (n=50)	CI1 (n=30)	CI2 (n=30)	CI3 (n=30)
Glucose, mol/l	11,75± 0,58	11,3± 0,55	12,8± 0,53^{++}	12,4± 0,61	13,1± 0,59	14,6± 0,57^{++}	9,5± 0,56	10,4± 0,55	11,2± 0,51^{+}	4,2± 0,04	4,5± 0,04	4,7± 0,04
IRI, mcU/ml	2,36± 0,12**	2,05± 0,10°°	1,98± 0,10^{++}	1,73± 0,08**	1,48± 0,07°°	1,2± 0,06^{++}	4,28± 0,11	4,27± 0,14	4,25± 0,12	4,51± 0,04	4,48± 0,04	4,52± 0,04
C-peptide, pmol/l	246,2± 17,3	244,4± 17,2	247,4± 16,4	187,5± 15,3	178,4± 16,8	165,8± 12,7	347,3± 14,4	348,4± 13,2	350,0± 17,3	339,4± 16,4	340,2± 15,1	341,5± 14,9
Hb_{A1c}, %	10,35± 0,12*	10,44± 0,14°	10,36± 0,11^{+}	12,02± 0,22**	11,84± 0,23°°	14,78± 0,25^{++}	9,77± 0,12**	9,75± 0,15°°	9,79± 0,14^{++}	4,86± 0,12^{x}	4,88± 0,13^{x}	4,65± 0,14^{x}
Lactate, mmol/l	1,45± 0,07	1,48± 0,07	1,51± 0,07	1,47± 0,07	1,49± 0,07	1,52± 0,07	1,44± 0,07	1,47± 0,07	1,50± 0,07	1,42± 0,01	1,43± 0,01	1,41± 0,01
Pyruvic acid, µmol/l	58,3± 2,86	61,3± 3,00	64,0± 3,14	59,6± 2,92	63,4± 3,11	66,9± 3,28	58,8± 2,88	61,4± 3,01	64,2± 3,15	57,4± 0,52	57,2± 0,51	57,6± 0,52
Lactate/ Pyruvic acid	0,024	0,024	0,023	0,024	0,023	0,022	0,024	0,023	0,023	0,024	0,025	0,024

*Note - **p<0.01; * p<0.05 compared to similar indicators in control group CI1; °°p<0.01; °p<0.05 compared to control group CI2; ++ p<0.01; + p<0.05 compared to control group CI3*

Table 2

Indicators of lipid and protein metabolism in the blood of rats with experimental diabetes

Index	Alloxan diabetes			Streptozotocin diabetes			Dithizone diabetes			Control		
	A1 (n=50)	A2 (n=50)	A3 (n=50)	S1 (n=50)	S2 (n=50)	S3 (n=50)	D1 (n=50)	D2 (n=50)	D3 (n=50)	CI1 (n=30)	CI2 (n=30)	CI3 (n=30)
FFA, µmol/l	0,43± 0,02	0,49± 0,02	0,53± 0,03	0,44± 0,02	0,51± 0,02	0,58± 0,03	0,45± 0,02	0,49± 0,02	0,52± 0,03	0,40± 0,004	0,41± 0,004	0,42± 0,004
Total cholesterol, mg/dl	213,6 ± 9,78	215,3± 10,01	219,2± 10,10	215,3± 9,91	219,4± 10,26	221,3± 10,35	223,3± 9,77	229,8± 9,92	231,6± 10,03	196,2± 1,78	197,4± 1,79	199,8± 1,79
TG, mg/dl	137,3 ± 1,82**	141,2± 0,96°°	148,6± 0,17^{++}	144,2± 1,87*	158,0± 0,11°°	158,5± 0,61^{++}	152,8± 0,89**	159,1± 0,14°°	162,3± 0,15^{++}	97,4± 0,88	98,3± 0,88	97,6± 0,88
HDL cholesterol, mg/dl	44,4± 2,32	42,2± 2,21	40,1± 2,11^{+}	39,6± 2,28	37,3± 2,17	32,5± 2,08^{+}	45,1± 2,36	43,4± 2,22	41,3± 2,12^{+}	59,4± 0,44	58,8± 0,44	59,1± 0,44
LDL-cholesterol, mg/dl	162,2 ± 0,87	164,3± 7,07*	171,5± 7,28^{+}	171,1± 6,91*	175,3± 7,12°	179,2± 7,31^{+}	160,6± 6,89	163,4± 7,03	167,5± 7,23^{+}	138,2± 1,24	139,1± 1,25	138,8± 1,25
VLDL cholesterol,	22,4± 1,00	23,6± 1,16	25,7± 1,26^{+}	25,6± 1,01	28,9± 1,17°	29,9± 1,27^{++}	22,1± 1,03	24,3± 1,19°	26,2± 1,28^{+}	19,3± 0,17	19,5± 0,18	19,8± 0,18

mg/dl												
Atherogenic index	3,6± 0,15***	3,8± 0,17°°°	3,9± 0,19^{++}	3,7± 0,16**	3,8± 0,18°°°	3,9± 0,19^{++}	3,5± 0,16**	3,7± 0,18°°°	3,9± 0,19^{+++}	0,89± 0,02	0,92± 0,02	0,88± 0,02
Apo A-1, mg/dl	140,1 ± 6,96	138,4± 6,93	136,2± 6,87	136,3± 6,92	131,2± 6,82	128,4± 6,78	141,8± 6,95*	137,6± 6,89	135,5± 6,84	142,3± 1,28	143,1± 1,29	143,3± 1,29
Apo B, mg/dl	118,2 ± 5,1	122,5± 5,2	129,4± 4,9	120,8± 5,2	129,4± 4,7	132,6± 6,1	118,8± 5,6	118,4± 5,1	124,1± 5,6	115,4 ±1,04	116,3± 1,05	116,8± 1,05
Total protein, mmol/l	67,3± 3,30	66,2± 3,24	65,8± 3,22	67,1± 3,29	65,4± 3,20	65,2± 3,19	67,0± 3,28	65,5± 3,21	65,3± 3,20	69,3± 0,62	68,8± 0,62	68,7± 0,62
Albumin, mmol/l	46,9± 2,30	46,4± 2,27	45,8± 2,24	46,3± 2,27	44,3± 2,17	43,2± 2,12	45,5± 2,23	45,1± 2,21	44,8± 2,20	48,3± 0,43	48,5± 0,44	48,8± 0,44
AST, nmol/s.l	17,5± 0,86	17,4± 0,85	17,8± 0,87	17,9± 0,88	18,0± 0,88	18,3± 0,90	17,7± 0,87	17,9± 0,88	18,0± 0,88	17,2± 0,15	17,4± 0,16	17,5± 0,16
ALT, nmol/s.l	16,4± 0,80	16,5± 0,81	16,8± 0,82	16,7± 0,82	17,1± 0,84	17,3± 0,85	16,8± 0,82	17,0± 0,83	17,2± 0,84	16,4± 0,15	16,2± 0,15	16,0± 0,14
*Note - ***p<0.001; **p<0.01; * p<0.05 compared to similar indicators in the control group CI1; °°°p<0.001; °°p<0.01; °p<0.05 compared to control group CI2; +++ p<0.001; ++ p<0.01; + p<0.05 compared to control group CI3*												

Similehe level of total cholesterol in intact animals (196.2 – 199.8 mg/dl) and in groups with alloxan and streptozotocin diabetes, a slight increase in this indicator to 221.3 mg/dl was determined. The total cholesterol index increased by 1.16 times in animals of group D3 in comparison with group CI3. A significant difference in the level of triglycerides in animals with experimental diabetes from that of intact animals was revealed. Thus, the level of triglycerides in subgroup A3 ($p<0.01$) was in 1.49 times higher compared to group CI3. The level of triglycerides in animals of group S3 ($p<0.01$) was in 1.59 times higher; and this index was in 1.66 time higher in group D3 ($p<0.01$) in comparison with the control group CI3.

A reliable ($p<0.05$) decrease in HDL content was noted in experimental animals of group A3 – in 1.47 time, in group S3 – in 1.81 time, in group D3 – in 1.43 time compared with group CI3.

The level of LDL-cholesterol in animals of groups A2 and A3 was significantly ($p<0.05$) increased by 1.20 and 1.23 times, respectively, compared with groups CI2 and CI3. Animals with streptozotocin diabetes showed an increase in LDL-cholesterol by 1.23-1.29 time ($p<0.05$), in animals of the D3 group – by 1.21 time in comparison with CI3 ($p<0.05$).

The identical characteristic dynamics was observed in the indicator of VLDL-cholesterol. In animals of the A3 group, this indicator was 1.29 times significantly ($p<0.05$) higher than in the CI3 group. A significant ($p<0.05$) increase in VLDL cholester-l was detected in animals with streptozotocin diabetes (groups C2 and C3) by 1.45 and 1.51 times, respectively, compared with CI2 and CI3 groups. A significant increase in this indicator was found only in animals with dithizone diabetes of groups D2 and D3 ($p<0.05$) – by 1.24 and 1.32 times compared with CI2 and CI3 groups.

The atherogenic index in intact animals was in the range of 0.88-0.92, while in animals of groups A3, S3 and D3 this indicator was significantly ($p<0.001$) higher by 4.43 times.

Sufficiently high background levels of Apo A-l (142.3 mg/dl, 143.1 and 143.3 mg/dl) and low levels of Apo B (115.4, 116.3 and 116.8 mg/dl) were noted in animals of control groups CI1, CI2 and CI3. Comparing these values with group A3, it is seen that the level of Apo A-1 decreased to 136.2 mg/dl, and the level of Apo B increased to 129.4 mg/dl. Apo A-1 levels decreased to 128.4 mg/dl in animals of subgroup S3, while Apo B level increased to 132.6 mg/dl. The level of Apo A-1 was 135.5 mg/dl in animals of subgroup D3, the level of Apo B was 124.1 mg/dl (Table 2).

A negligible decrease in total protein values, depending on the raise in the observation period, was found in experimental animals with different models of diabetes in groups A3, S3 and D3. A slight increase in AST and ALT was registered in animals with streptozotocin and dithizone diabetes.

A decline in the level of hormones, depending on the increase in the observation period, was found in experimental animals. Thus, the level of CTH in the blood of animals with alloxan diabetes subsidenced in animals of group A1 to 37.1 pM/l, in animals of group A2 – to 36.7 pM/l, in animals of group A3 – to 35.4 pM/l. A decrease in the level of corticotropin in the blood was noted in animals with dithizone diabetes of group D1 – up to 37.6 nm/l, group D2 – up to 36.9 nm/l, group D3 – up to 36.2 nm/l. Whereas in animals

with streptozotocin diabetes, ACTH values were minimal compared to animals with alloxan and dithizone diabetes and ranged from 35.3 to 32.2 pM/l (Table 3).

The level of adrenaline in the blood of the control groups was 2.14-2.16 nM/l, while this index in all experimental animals was significantly high ($p<0.01$). Thus, adrenaline decreased from 5.43 to 3.12 nm/l (A3 is 1.45 times higher than CI3) in animals of the groups with alloxan diabetes. The level of adrenaline in animals with streptozotocin diabetes decreased from 5.31 to 3.07 (1.42 times more compared to CI3). The level of adrenaline in animals with dithizone diabetes decreased from 5.37 to 3.03 (1.4 times more than CI3).

Analogous dynamics was noted in the study of the level of norepinephrine. Thus, low rates at 24 months of observation were established in groups of animals A3, S3 and D3. Whereas the level of norepinephrine was significantly ($p<0.05$) high at 6 and 12 months of observation. Thus, this indicator increased in animals of subgroup A2 by 1.86 times, in animals of subgroup S2 – by 1.85 times, in animals of subgroup D2 – by 1.80 times compared with group CI2.

Cortisol levels in animals of control groups (18.4-18.6 μM/l) were compared. Thus, cortisol level was 18.2 μM/L in animals with alloxan diabetes at 6 months of observation. This decreased to 15.8 μM/l (A2) after 12 months of follow-up and recovered to 17.3 μM/l after 24 months of follow-up (A3). The cortisol level tended to decrease as the observation period increased in animals with streptozotocin (14.5; 13.3; 12.4 μM/l) and dithizone (18.1; 17.4; 15.5 μM/l) experimental diabetes. The synthesis of cortisol was reduced by 1.5 times in the animals of group S3 compared to group CI3.

Table 3

Indicators of blood hormones in rats with experimental diabetes

Index	Alloxan diabetes			Streptozotocin diabetes			Dithizone diabetes			Control		
	A1 (n=50)	A2 (n=50)	A3 (n=50)	S1 (n=50)	S2 (n=50)	S3 (n=50)	D1 (n=50)	D2 (n=50)	D3 (n=50)	CI1 (n=30)	CI2 (n=30)	CI3 (n=30)
ACTH, pM/l	37,1 ± 1,3	36,7 ± 1,7	35,4 ± 1,3	35,3 ± 1,6	33,7 ± 1,5	32,2 ± 1,4	37,6 ± 1,8	36,9 ± 1,3	36,2 ± 1,6	37,4 ± 0,3	38,2 ± 0,2	37,9 ± 0,3
Adrenaline, nM/l	5,43 ± 2,4***	4,88 ± 0,22°°°	3,12 ± 0,15^{++}	5,31 ± 0,23**	4,59 ± 0,22°°	3,07 ± 0,12	5,37 ± 0,23**	4,61 ± 0,19°°°	3,03 ± 0,13^{++}	2,14 ± 0,02	2,16 ± 0,01	2,15 ± 0,02
Norepinephrine, nM/l	4,15 ±2,05*	3,88 ± 0,12°	2,52 ± 0,11^{+}	4,03 ± 0,19*	3,86 ± 0,17°	2,92 ± 0,13	4,08 ± 0,18*	3,76 ± 0,15°	2,66 ± 0,12^{+}	1,92 ± 0,02	2,08 ± 0,02	2,11 ± 0,02
Cortisol, μM/l	18,2 ± 0,88	15,8 ± 0,75	17,3 ± 0,7	14,5 ± 0,8	13,3 ± 0,7	12,4 ± 0,6	18,1 ± 0,87	17,4 ± 0,81	15,5 ± 0,6	18,6 ± 0,2	18,5 ± 0,2	18,4 ± 0,15
Glucagon, ng/ml	23,3 ± 1,4	22,5 ± 1,3	21,3 ± 1,2^{+}	20,1 ± 1,3	17,2 ± 1,2	16,9 ± 1,1^{+}	24,1 ± 1,4	22,4 ± 1,3	20,6 ± 1,1	26,41 ± 0,2	26,39 ± 0,3	26,42 ± 0,2
STH, ng/ml	1,48 ± 0, 06**	2,32 ± 0,11°°	3,07 ± 0,14^{++}	1,21 ± 0,04**	1,96 ± 0,08°°	2,14 ± 0,08^{++}	1,98 ± 0,09**	2,65 ± 0,12°°	3,14 ± 0,14^{+++}	5,5 ± 0,05	5,3 ± 0,05	5,2 ± 0,04
TSH, mcU/ml	3,72 ± 0,11**	5,76 ± 0,11°°	8,79 ± 0,13^{++}	1,16 ± 0,09**	1,41 ± 0,1°°	1,67 ± 0,12	3,55 ± 0,11	4,68 ± 0,13	5,70 ± 0,12	2,25 ± 0,01	2,27 ± 0,02	2,28 ± 0,01
T_3, nM/l	7,43 ± 0,44	7,02 ± 0,45	6,82 ± 0,43	6,44 ± 0,42	6,56 ± 0,44°°	4,73 ± 0,39^{++}	7,32 ± 0,46	6,76 ± 0,38	6,44 ± 0,41	9,24 ± 0,1	9,24 ± 0,09	9,26 ± 0,08
T_4,nM/l	132 ± 7,5	130 ± 6,9	128 ± 6,2	118 ± 6,5	114 ± 5,6	103 ± 5,7	131 ± 6,2	133 ± 6,9°	132 ± 5,9	135 ± 1,5	134 ± 1,1	133 ± 1,1

*Note - ***-p<0.001; **-p<0.01; *-p<0.05 compared with similar indicators in control group CI1; °°°-p<0.001; °°-p<0.01; °-p<0.05 compared with group CI2; +++ - p<0.001; ++ - p<0.01; + - p<0.05 compared with control group CI3*

The study of the contrainsular hormone (glucagon) in experimental animals showed that the level of glucagon was reduced, especially in streptozotocin diabetes in animals of group S3 – significantly ($p<0.05$) in 1.56 times compared with group CI3. A slight decrease was registered in animals with alloxan and dithizone diabetes.

We noted significant differences in the content of growth hormone in animals with experimental diabetes. If in intact animals its level was 5.5-5.2 ng/ml, then as the period of observation of diabetes increased, the level of growth hormone increased. So, at 6 months of observation, there were significantly ($p<0.01$) the lowest levels of GH in group A1 (in 3.71 times), S1 (in 4.54 times), D1 (in 2.77 times); A2 (in 2.28 times), S2 (in 2.7 times), D3 (in 2.0 times) and A3 (in 1.69 times), S3 (in 2.42 times) and D3 (in 1.56 times) compared with intact animals (Fig. 2).

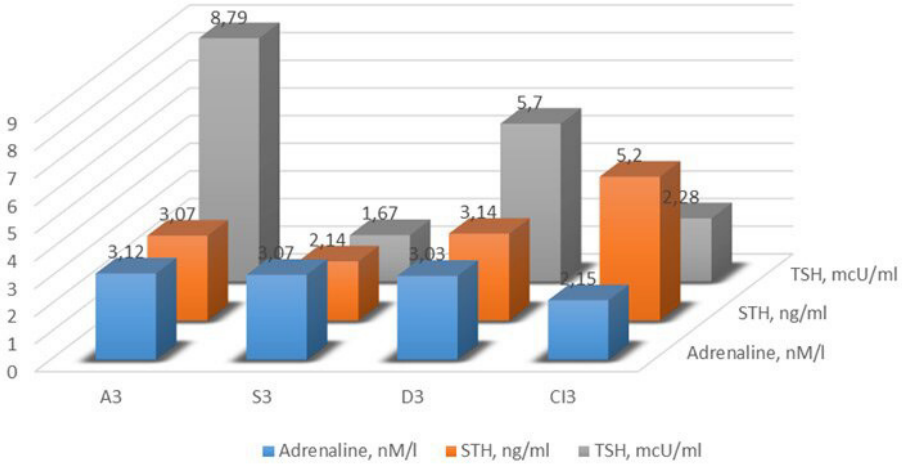

Figure 2. Indices of hormones in the blood of rats with experimental diabetes

The level of TSH in the blood of animals of groups CI1, CI2 and CI3 was equal to 2.25-2.28 mcU/ml, respectively. The level of TSH significantly ($p<0.01$) increased in animals with alloxan diabetes depending on the observation period: in animals of group A1 – in 1.65 times compared with CI1; in animals of group A2 – in 2.53 times compared with CI2; in animals of group A3 – in 3.85 times compared with CI3. The TSH index decreased in animals with streptozotocin diabetes as the observation period increased in comparison with intact animals. Thus, the level of TSH significantly ($p<0.01$) decreased in 1.93 times in animals of subgroup S1 compared to the CI1 group; in animals of subgroup S2, the level of TSH decreased ($p<0.01$) in 1.6 times compared to group CI2, in animals of subgroup S3 this indicator decreased in 1.36 times compared to group CI3. In animals with dithizone diabetes, the TSH index increased as the observation period increased, but was lower than in animals with alloxan diabetes.

The level of T3 in animals with alloxan diabetes was slightly lower compared to intact groups. The level of T3 hormone in experimental animals decreased as the observation period increased. The lowest level was registered in animals with streptozotocin diabetes in subgroups S1 ($p<0.01$) – in 1.66 times and S2 – in 1.95 times in comparison with groups CI1 and CI2 (Fig. 2). The level of T4 in control animals in groups CI1, CI2 and CI3 was in the range of 133-135 nM/l, but in animals with experimental diabetes it decreased with an increase in the observation period. In all animals with experimental models, T4 index was less than in intact animals, but in animals of group S3 it was in 1.3 times less than in group CI3.

Discussion

Conclusion, we can assume that the experimental data helped to reveal many aspects of the etiology and pathogenesis of the early stages of diabetes mellitus. In animals with alloxan and streptozotocin diabetes in the stage of pre-diabetes, latent diabetes and in the early periods of obvious diabetes, a decrease in insulin secretion and the development of absolute insulin deficiency were demonstrated. Animals with dithizone diabetes develop relative insulin deficiency. Hormonal insulin antagonists can cause both the development of diabetes mellitus in animals and the transition of prediabetes and latent diabetes to overt.

So, in alloxan and streptozotocin models of diabetes, carbohydrate balance disorders identical to patients with diabetes mellitus type 1 were revealed, and in dithizone diabetes, changes characteristic of patients with diabetes mellitus type 2 were revealed. In animals with streptozotocin diabetes, a significant ($p<0.01$) raise in glycemia in 3.10 times, glycated hemoglobin in 3.16 times, IRI decrease ($p<0.01$) in 3.76 times and C-peptide in 2 .05 times compared with intact animals.

Whereas, expressed lipid metabolism disorders was detected in alloxan, streptozotocin and, especially, dithizone diabetes (after 24 months of observation). It is reflected in a significant ($p<0.01$) increase of triglycerides levels in 1.66 times, atherogenic index ($p< 0.01$) – in 4.43 times and a decrease ($p<0.05$) of HDL levels – in 1.43 times compared with intact group CI3, as well as increase in FFA, total cholesterol, LDL, VLDL.

The activity of the sympathoadrenal system was increased in animals with alloxan and dithizone diabetes. It was confirmed by a significant ($p<0.01$) increase in the level of adrenaline, norepinephrine, as well as

a decrease in the functional activity of the pituitary gland (decrease in the level of growth hormone ($p<0.01$) and CTH), adrenal cortex and α-cells of the pancreas. A more pronounced inhibition of the endocrine glands was registered in animals with streptozotocin diabetes.

Conclusions

To sum up the functional activity of the pituitary gland (growth hormone level and CTH ($p<0.01$), the adrenal cortex and pancreatic α-cells was reduced ($p<0.01$) in animals with alloxan and dithizone diabetes. The activity of the sympathoadrenal system was increased in the indicated animals. These facts were confirmed by reliable ($p<0.01$) increase of the level of adrenaline, norepinephrine in 1.42 times. More expressed inhibition of endocrine glands was registered in animals with streptozotocin diabetes. Inhibition of the functional activity of adrenal and thyroid hormones in animals with streptozotocin diabetes leads to depletion of the structural and functional reserve. In alloxan and dithizone diabetes, the indicators of adrenal and thyroid hormones had a phase character: from the period of 6 months – signs of depression, followed by adaptive restructuring and resistance by 12 months and signs of some compensatory activation by 24 months of observation.

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Аллоксан, стрептозотоцин және дитизон қант диабетімен эксперименттік жануарлардағы гормоналды-метаболикалық бұзылуларды клиникалық және зертханалық бағалау

Эксперименттік диабетологияда негізгі орынды қант диабетінің химиялық модельдері алады. Бұл модельдерде әртүрлі агенттер ұйқыбезі аралдарының тініндегі β-жасушаларына таңдамалы түрде әсер етеді. Жануарлардағы гормоналды және метаболикалық бұзылулардың патогенетикалық механизмдері аллоксан, стрептозотоцин және дитизон қант диабеті модельдерінде зерттелді. Тәжірибе үшін Вистар тұқымының еркек егеу құйрықтары қолданылды. Эксперименттік қант диабетінің үш моделі жасалды. Бақылау топтарының уақыты (6, 12, 24 ай) және саны бойынша бірдей болды.

Липидтер мен ақуыз алмасуын зерттеудің зертханалық-биохимиялық әдістерінің кешені қолданылып, сарысу ферменттері де анықталды. Аллоксан мен стрептозотоцинді енгізгеннен кейін эксперименттік жануарлардағы гликемия деңгейі 13,4-тен 18,6 ммоль/л-ге дейін ауытқиды, глюкозурия, полидипсия, полифагия байқалды. Стрептозотоцин қант диабеті бар жануарларда бақылау тобына қарағанда гликемияның 3,10 есе, гликирленген гемоглобиннің — 3, 16 есе, IRI — 3, 76 есе және С–пептидтің – 2,05 есе ($p<0,01$) жоғарылауы байқалды. Аллоксан, стрептозотоцин және әсіресе дитизон қант диабетінде липидтер алмасуының ауыр бұзылыстары анықталды (24 айлық бақылау). Бұл триглицеридтер деңгейінің 1,66 есе, атерогенділік индексінің ($p<0,01$) 4,43 есе және ЖТЛП деңгейінің ($p<0,05$) СИЗ бұзылмаған тобымен салыстырғанда 1,43 есе, сондай–ақ МҚҚ, ЖХС, ТТЛП, ТӨТЛПайтарлықтай ($p<0,01$) жоғарылауы байқалды. Аллоксан және дитизон қант диабеті бар жануарларда гипофиздің функционалдық белсенділігі төмендеді (өсу гормоны мен КТГ деңгейі ($p<0,01$), бүйрек үсті безінің қыртысы және ұйқы безінің α-жасушалары ($p<0,01$), симпатоадреналин жүйесінің белсенділігі жоғарылайды, бұл адреналин, норадреналин деңгейін жоғарылату арқылы ($p<0,01$) — айтарлықтай 1,42 есеге расталды. Стрептозотоцин қант диабеті бар жануарларда ішкі секреция бездерінің жұмысының айқын тежелуі байқалды.

Кілт сөздер: эксперименттік аллоксан, стрептозотоцин және дитизон қант диабетінің модельдері, гликирленген гемоглобин, С-пептид, липидті және ақуыз алмасу көрсеткіштері, кортикотропты гормон, адреналин, норадреналин, кортизол, бос гидрокортизон көрсеткіштері, глюкагон, инсулин.

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Клинико-лабораторная оценка гормонально-метаболических нарушений у экспериментальных животных с аллоксановым, стрептозотоциновым и дитизоновым диабетом

Основное место в экспериментальной диабетологии занимают химические модели диабета. В них различные агенты избирательно воздействуют на *β*-клетки в ткани островков поджелудочной железы. Патогенетические механизмы гормональных и метаболических нарушений у животных изучались на моделях аллоксанового, стрептозотоцинового и дитизонового диабета. Для эксперимента использовали самцов крыс линии Вистар. Были созданы три модели экспериментального диабета. Группы были идентичны по времени наблюдения (6, 12, 24 месяца) и количеству. Применялся комплекс лабораторно-биохимических методов исследования липидного и белкового обмена. Также определяли сывороточные ферменты. Уровень гликемии у экспериментальных животных после введения аллоксана и стрептозотоцина колебался от 13,4 до 18,6 ммоль/л, замечены глюкозурия, полидипсия, полифагия. У животных со стрептозотоциновым диабетом наблюдалось достоверное ($p<0,01$) увеличение гликемии в 3,10 раза, гликированного гемоглобина — в 3,16 раза, снижение ($p<0,01$)ИРИ — в 3,76 и С-пептида — в 2,05 раза по сравнению с интактными животными. Тяжелые нарушения липидного обмена были выявлены при аллоксановом, стрептозотоциновом и особенно дитизоновом диабете (24 месяца наблюдения). Это выразилось в значимом ($p<0,01$) повышении уровня триглицеридов — в 1,66 раза, индекса атерогенности ($p<0,01$) — в 4,43 и снижении ($p<0,05$) уровня ЛПВП — в 1,43 раза по сравнению с интактной группой КИЗ, а также обнаружено увеличение СЖК, ОХС, ЛПНП, ЛПОНП. У животных с аллоксановым и дитизоновым диабетом была снижена функциональная активность гипофиза (уровень гормона роста и СТГ ($p<0,01$), коры надпочечников и α-клеток поджелудочной железы ($p<0,01$), повышена активность симпатоадреналовой системы, что было подтверждено значительным ($p<0,01$), за счет повышения уровня адреналина, норадреналина — в 1,42 раза. Более выраженное подавление работы желез внутренней секреции отмечено у животных со стрептозотоциновым диабетом.

Ключевые слова: модели экспериментального аллоксанового, стрептозотоцинового и дитизонового диабета, гликированный гемоглобин, *С*-пептид, показатели липидного и белкового обмена, показатели кортикотропного гормона, адреналина, норадреналина, кортизола, свободного гидрокортизона; глюкагон, инсулин.