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Histological Changes in Pancreatic Islets of Animals with Experimental Diabetes Caused by Xanthurenic Acid under Condition of Suppression of Its Endogenous Synthesis

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Long-term administration of pyridoxine to rats kept on a diabetogenic diet stimulating endogenous synthesis of xanthurenic acid resulted in minimal glycemia, less pronounced decrease in insulin content in β-cells, and more intensive excretion of xanthurenic acid with urine. Histological changes were observed in 23% pancreatic islets, whereas in rats not treated with pyridoxine, destruction and necrosis of 40-45% β-cells were found in 38% of studied islets.

Key Words: *xanthurenic diabetes; β-cells; Zn^{2+} ions; pyridoxine*

Xanthurenic acid (4,8-dihydroxyquinoline-2-carbonic acid) (XA) is a diabetogenic complex-forming derivative of 8-oxyquinoline. Of 17 representatives of this group, only XA is actively synthesized in animals and aged people in triptophan metabolism disturbances combined with long-term pyridoxine deficit [1,2]. The key mechanism of the diabetogenic effect of XA (similar to other 8-oxyquinoline derivatives) is the formation of toxic complexes with Zn^{2+} ions [14,15] present in high concentrations in β-cells cytoplasm, which leads to their selective damage. These changes are followed by damage to the walls of blood capillaries in islets and can aggravate the course of diabetes [9]. Zn^{2+} ions are involved in the formation of the proinsulin hexamer (insulin storage form), reduce insulin solubility, and participate in microcrystallization of the hormone with its accumulation in β-granules [3,6,8]. Destruction of these granules then leads to β-cell destruction under the influence of diabetogenic complex-forming substances including 8-oxyquinoline derivatives [3]. Pancreatic islets of rats, mice, rabbits, dogs, horses, birds, some species of fishes, hamsters, pigs, and humans contain Zn^{2+}ions in high concentrations. There are three possible ways for prevention of diabetes induced by complex-forming substances: 1) almost complete elimination of Zn^{2+} ions from β-cells prior to exposure to the chelator in the diabetogenic dose, 2) preliminary binding of Zn^{2+} ions by non-diabetogenic complex-forming substances, 3) suppression of endogenous XA synthesis via pyridoxine supplementation. The first two ways are effective under the experimental conditions, but in real diabetes, they are ineffective in XA control, because under natural conditions it is synthesized gradually during long periods.

Here we studied the possibility preventing β-cell death via suppression of endogenous XA synthesis.

MATERIALS AND METHODS

The study was performed on albino Wistar rats (n=34) weighting 158-169 g and neonatal Lewis rats (n=20). Wistar rats were kept on a diabetogenic diet for 108-116 days [1] stimulating endogenous synthesis of XA

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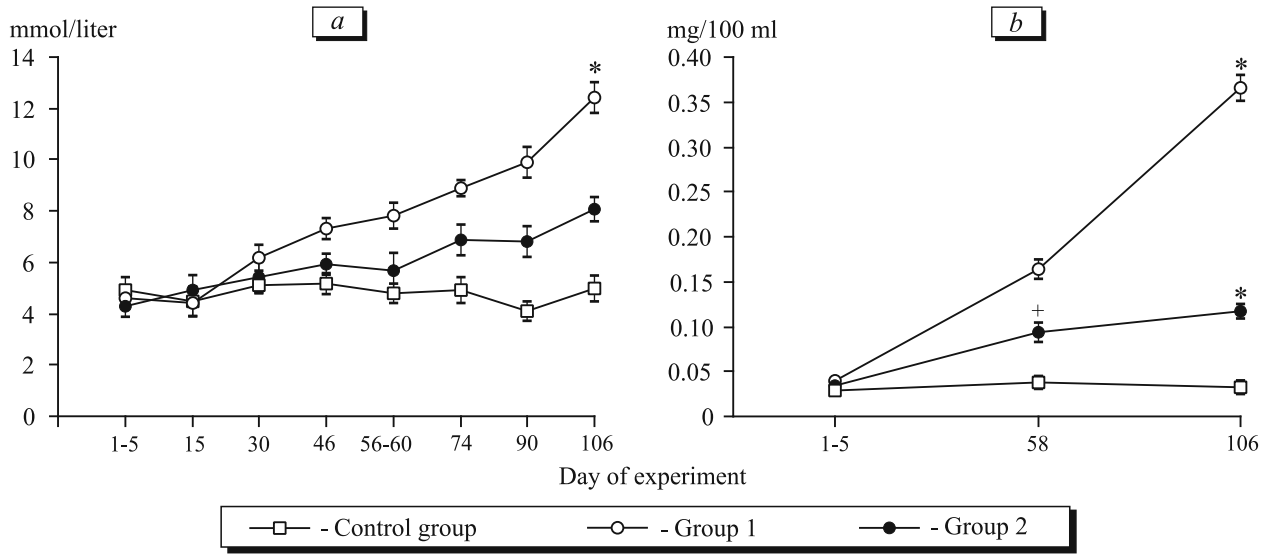

Fig. 1. Blood glucose (*a*) and xanthurenuria (*b*) in rats of the experimental groups. *$p<0.001$ in comparison with control group at the same

(52% starch, 15% butter, 22% casein, 3% yeast, 5% sugar, and 3% salt). Group 1 (n=12) was kept only on the diet, while group 2 (n=22) intraperitoneally received pyridoxine starting from week 3 of diet (150 mg/kg, 1 time per 2 days for 2 weeks and then 100 mg/kg by the same scheme until the end of the experiment). Control group (n=10) consisted of the intact rats. Glucose concentration in the blood was controlled weekly; XA excretion with urine was evaluated 3 times: on days 1-5, 56-60, and 104-108.

Pancreas specimens were fixed in Bouin fixative. The following histological and histochemical techniques for visualization of insulin and zinc in β-cells were used: 1) immunohistochemistry (DAKO kits), aldehyde fuchsin (Avocado Chemical Co) [10], diethylpseudoisocyanine (Serva) [7,13]. Zn^{2+} ions were stained with highly specific luminescent reagent (8-para(toluolsulphonilamino)quinoline (8TCQ) [12]. Insulin and zinc concentrations in β-cells were quantitatively evaluated using a histofluorometric method by the coefficient of fluorescence of the diethylpseudoisocyanine-stained samples: K=IF1/IF2 (for zinc ZF1/ZF2), where IF1 is fluorescence intensity (mA) of β-cells stained for insulin and IF2 is the intensity of exocrine cell fluorescence [4].

For the analysis of the direct influence of XA on β-cells, pancreatic islets were isolated as described previously [11]. In brief, the pancreas from rat pups was disaggregated in 2% collagenase solution (Fluka; 3×5 min), fractionated in the dextran density gradi-

TABLE 1. Dynamics of Blood Glucose Levels and Xanthurenutia in Animals Kept on Diabetogenic Diet

Day of study	Intact rats		XA-induced diabetes	
	glucose, mmol/liter	xanthurenuria, mg/100 ml	glucose, mmol/liter	xanthurenuria, mg/100 ml
1-5	4.9±0.5	0.029±0.005	4.6±0.4	0.039±0.003
15	4.5±0.6		4.4±0.5	
30	5.1±0.3		6.2±0.5	
46	–		7.3±0.4	
56-60	4.8±0.4	0.038±0.007	7.8±0.5	0.164±0.011
74	4.9±0.5		8.9±0.3	
90	9.9±0.6		4.1±0.4	
106	5.0±0.5	0.033±0.005	12.4±0.6*	0.366±0.014*

Note. *$p<0.001$ in comparison with the corresponding parameter in intact group.

TABLE 2. Dynamics of Blood Glucose Levels and Xanthurenutia in Animals Kept on Diabetogenic Diet and Treated with Pyridoxine

Day of study	Blood glucose, mmol/liter		Xanthurenuria, mg/100 ml	
	in 19 rats	in 3 rats	in 19 rats	in 3 rats
1-5	4.3±0.4	4.7±0.5	0.035±0.005	0.041±0.006
15	4.9±0.6	5.2±0.4		
30	5.4±0.6	4.9±0.4		
46	5.9±0.4	6.4±0.6		
56-60	5.7±0.7	6.3±0.4	0.093±0.011*	0.144±0.008*
74	6.9±0.6	7.9±0.7		
90	6.8±0.6	9.8±0.5		
106	8.1±0.5	10.6±0.6	0.118±0.008*	0.226±0.011*

Note. *$p<0.05$ in comparison with the same parameter in 3 rats at the corresponding term.

ent (Pharmacia Fine Chemicals), washed in Hanks saline, and then cultured for 8 h in nutrient medium RPMI-1640 (Serva) with 5.5 mM glucose. Two experimental groups (250 units each) were incubated with 120 mkg/ml XA (Serva) for 1 h. After centrifugation, the islets were fixed in Bouin fixative for 15 min. Paraffin sections were stained using the same methods as for pancreatic tissue. The samples were examined by light and luminescent microscopy at λ_{abs}=360-370 nm. The concentration of the insulin and zinc in β-cells stained with aldehyde fuchin and 8-TCQ, and in the sections of isolated islets was evaluated by calculation of the K coefficient: k=AF1/AF2 (for zinc - ZF1/ZF2), where AF1 and AF2 are absorbtion and emmision intensities of fluorescence (ZF1 and ZF2) of the β-cells of the experimental and intact islets [4,5]. Insulin concentration was evaluated from a negative relationship: the higher light absorption, the higher insulin content in cells, while Zn^{2+} concentration was calculated from a positive relationship: higher fluorescence intensity of β-cells, the higher Zn^{2+} content in cells. Absorption and emission of intact β-cell fluorescence were taken as 1.

The data were processed by ANOVA (Student *t* test).

RESULTS

In 19 rats of group 2, glycemia was less pronounced than in group 1 controls starting from day 46 of the study (Fig. 1, *a*). In 3 rats of group 2, glucose level steadily increased starting from day 75 and attained 10.6±0.06 mmol/liter by day 106 (*i.e.* did not differ from the control).

Xanthurenuria significantly increased in both experimental groups starting form days 56-60, especially in group 1 animals, where it almost 3-fold surpassed the control level by the end of the experiment (Table 1, 2, Fig. 1, *b*).

In 3 rats of group 2, the level xanthurenuria 5-fold surpassed the initial level in this group, but was by 30% lower than in group 1.

TABLE 3. Insulin and Zn^{2+} Content in β-Cells in XA-Induced Diabetes and under Conditions of Suppression of Its Endogenous Synthesis (rel. units)

Experimental conditions	Insulin (IF1/IF2)	Insulin (IG1/IG2)	Zn^{+2} (ZFI1/ZFI2)
Intact animals	2.08±0.08	1.95±0.03	2.02±0.07
XA-induced diabetes (group 1)	1.28±0.05	1.18±0.04	1.21±0.03
Suppression of endogenous synthesis (group 2)	1.67±0.02*	1.58±0.02*	1.68±0.04*
Isolated intact islets (AF1/AF2)	1.00±0.03	–	1.00±0.04
Isolated islets after XA (ZF1/ZF2)	0.18±0.02	–	0.07±0.01

Note. *$p<0.001$ in comparison with the corresponding parameter in XA-induced diabetes.

Histological and histochemical study revealed a decrease in insulin stores and Zn^{2+} content in β-cells cytoplasm in both groups, which was more pronounced in animals with XA-induced diabetes and in islets exposed to direct influence of synthetic XA (Fig. 2, 3). In animals treated with pyridoxine for more than 2 months (2nd group), insulin concentration in β-cells was much higher than in rats with XA-induced diabetes (by 30% according to immunehistochemistry data and by 34% according to more sensitive luminescent diethylpseudoisocyanine method). In group 2 rats, the content of Zn^{2+} ions in β-cells was 38% higher than in group 2, and 23% lower than in intact rats (Table 3). Necrosis and destruction of β-cells were found in 38.0±6.6% islets from animals with XA-induced diabetes (group 1). In diabetes caused by intravenous injection of other complex-forming substances, 90-95% of islets were involved [3]. Destruction and necrosis were found in 40-45% islet area. Pyknosis of cell nuclei, hyperemia, and stasis were observed in islet capillaries. In group 2 animals, necrosis was revealed in 23.0±5.9% islets and occupied 19-25% islet area.

Thus, long-term pyridoxine administration under conditions of the diet stimulating endogenous synthesis of XA significantly reduced glucemia and XA excretion with urine (by more than 2 times) and increased insulin depots (by 30-38% more) and zinc concentration in β-cells.

In rats treated with pyridoxine, histological changes in the pancreatic islets were less prominent and were restricted to necrosis, destruction, nuclear picnosis, hyperemia and stasis in the islet vessels.

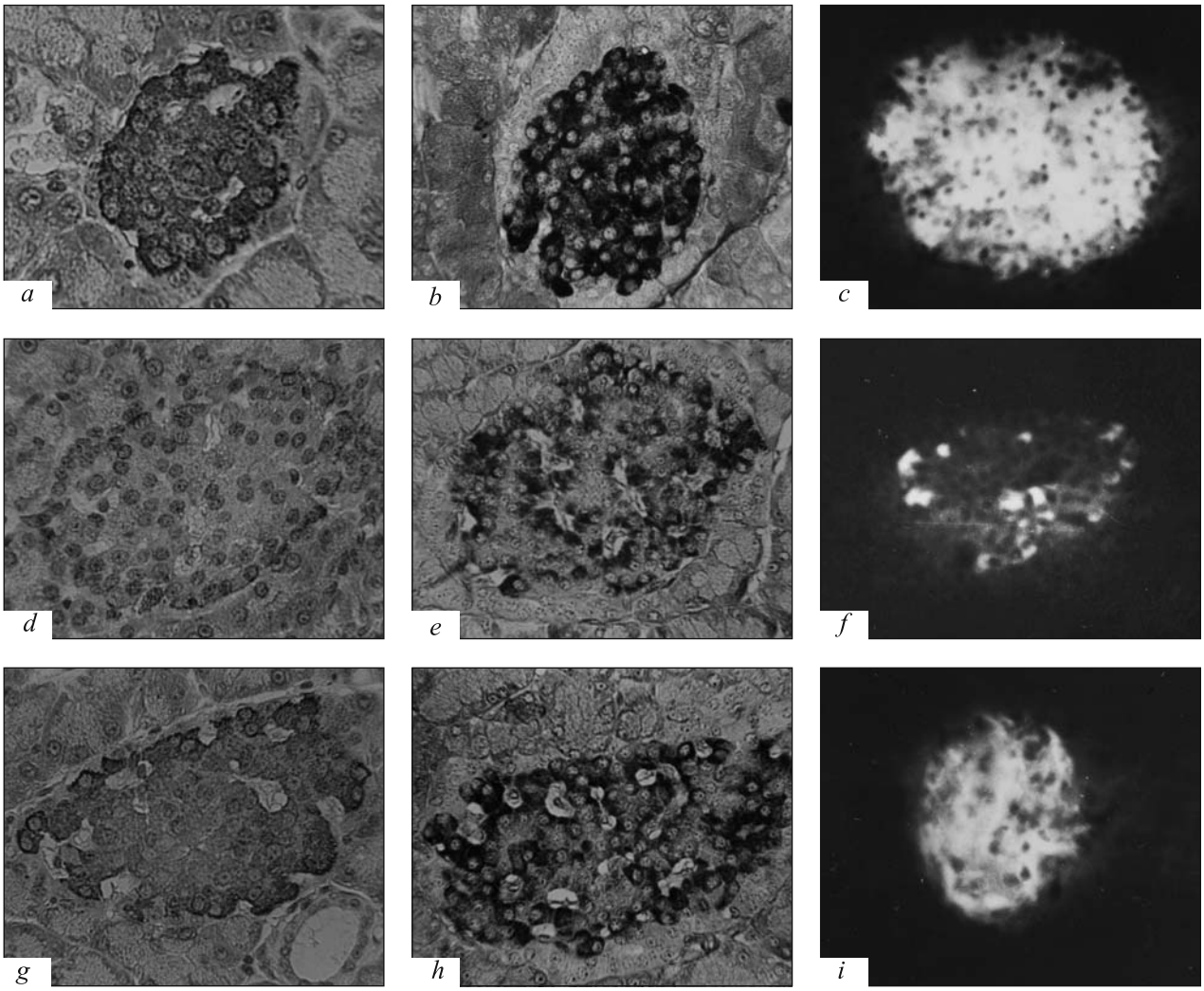

Fig. 2. Histostructure and content of insulin and Zn^{+2} in β-cells of pancreatic islets under normal conditions (*a-c*), in XA-induced diabetes (*d-f*), and after long-term pyridoxine treatment (*g-i*). Immunohistochemistry (*a, d, g*) and staining with aldehyde fuchsin (*b, e, h*): dark staining corresponds to higher insulin content. Luminescent 8TCQ staining for Zn^{2+} (*c*, *f*, *i*): bright fluorescence corresponds to high Zn^{2+} content (*c*, i), weak fluorescence (*f*): ×280 (*a*, *b*, *d*, *e, g, h*); ×160 (*c*, *f*, *i*). Insulin - (*a*, *b*, *d*, *e, g, h*); Zn^{2+} (*c*, *f*, *i*).

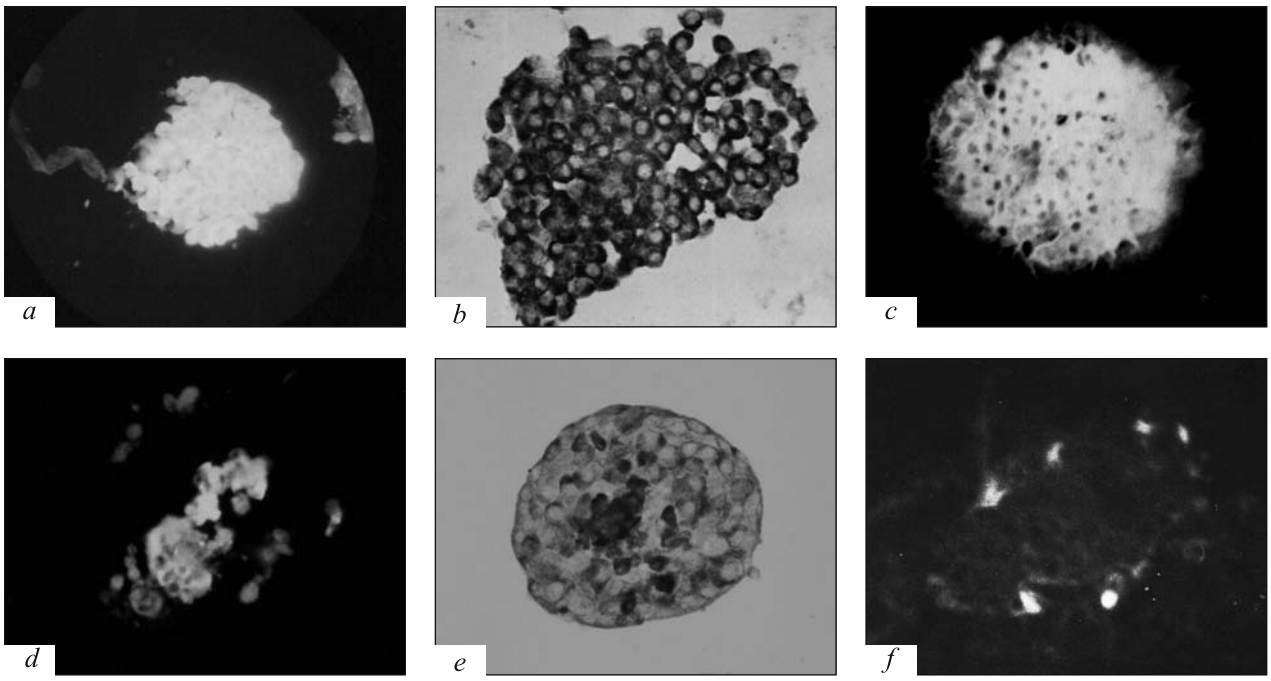

Fig. 3. Histostructure of isolated pancreatic islets and content of insulin and Zn^{+2} in β-cell cytoplasm under normal conditions (*a-c*) after incubation with XA (*d-f*). Diethylpseudoisocyanine staining (*a, d*): normal (*a*) and sharply reduced (*d*) insulin content. Luminescent 8TCQ staining for Zn^{2+} (*c*, *f*): bright fluorescence corresponds to high (*c*) and weak fluorescence to low (*f*) Zn^{2+} content. Aldehyde fuchsin staining (*b, e*): bright staining corresponds to high (*b*) and weak staining to low (*e*) insulin content. ×160 (*a*, *c*, *d*, *f*); ×280 (*b*, *e*).

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