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State of histostructure of liver and of exocrine pancreas tissues in experimental diabetes caused by diabetogenic metabolites of tryptophan

Authors showed new experimental data on the state of histostructure of Liver and Exocrine Pancreas tissue in experimental diabetes approached on conditions of development to human 2 type diabetes caused by 4,8-dihydroxyquinolin-2-carboxylic acid — the substance actively synthesized in elderly human, unlike all known today more than 30 diabetogenic substances. It is established that, at the same time with destruction of pancreatic B-cells that is a direct cause of diabetes, a multiple destructive changes are developed in a Liver and in Exocrine tissue of Pancreas. Authors suppose these changes which are not belong to direct causes of developed diabetes, but can aggravate diabetes considerably.

Key words: pancreatic islets, B-cells, liver, experimental diabetes, distrophya, necrosis, destruction of cells, diabetogenic chemicals, diabetogenic metabolites of tryptophan, hydropic degeneration.

Background

Experimental diabetes caused by selective damage of pancreatic B-cells by diabetogenic chemicals is very convenient model of diabetes which allowed to obtain a large number of scientific data on the reasons and mechanisms of development of diabetes for the last 70 years [1, 2].

However, models of diabetes which is selectively induced by chemicals, are belong to artificial models and has some serious shortcomings: 1) its developed not gradually as it most often happens in animals or human and is one-stage as result of death of B-cells within very short period; 2) almost all number of widely known diabetogenic chemicals have no relation as the reason to human diabetes because are not formed in organism and not delivered into outside; 3) diabetes caused by chemicals result destruction and death of B-cells only whereas diabetes developing at natural conditions, can be accompanied by changes in other tissues not only in Pancreas.

One of many diabetogenic chemicals as 4,8-dihydroxyquinolin-2-carboxylic acid (4,8DQC; Xanthurenic acid), a diabetogenic metabolit of Tryptophan, formed in animals and Human in deficiency of vit. B6 and abundance of fats that result change of Tryptophan metabolism from Serotonin way to Kynurenine [3, 4]. It is known that direct action of 4,8DQC result destruction and death of B-cells within short time as other diabetogenic derivatives of 8-oxyquinolin [5–12]. There are 2 ways for destruction of pancreatic B-cells using 4,8DQC: direct action of synthetic 4,8DQC on B-cells and gradual accumulation in organism of 4,8DQC similar on natural conditions of accumulation of this substance in human body. In such experimental conditions this is a question: except damage of B-cells what changes can be developed in other tissues which can have impact on diabetes development? Especially we interested by state of histostructure of liver due to its significant role in regulation of Glucose metabolism and in exocrine tissue of Pancreas.

Aim of work: to investigate state of histostructure of liver and exocrine Pancreas tissue in animals with diabetes caused by 4,8DQC.

Materials and Methods

Animals. 49 Rats Wistar 152–170 g were used. 1st group, 21 Rats contained 116–120 days on diabetogenic diet by Y.Kotake [2, 13]. Diet structure: starch — 52 %, casein — 22 %, butter — 15 %, sugar — 5 %, yeast — 3 %, mineral salts-3 %. Control of blood Glucose level and weight control weekly and of Xanturenuria (XAU) — monthly. Fixation of Pancreas tissue and of Liver in Bouin 24h. Paraffin sections 4 mcm were prepared by rotation microtome «Leica 2125». **Staining technologies**.Liver tissue: haematoxylin and eosin; haemalaune-eosin; staining of glycogen by SHIFF-method. Pancreas tissue: staining by aldehyde-fucshin method, insulin staining by immunohistochemical and diethylpseudoisocyanine technics [14, 15].

Results

Blood Glucose level was increased at 90th day containing on diet from 4,98±0,22 mM until 7,06±0,33 mM past 30 day on diet to 6,72±0,25 mM 70 days and to 9,27± 0,38* mM at 90th day (Table 1).

Concentration of 4,8-dihydroxyquinolin-2-carboxylic acid in the Urine was increased almost 10 times at 93rd day on diet comparatively with 5th day containing of animals on Diabetogenic diet (Table 2).

Table 1

Blood Glucose concentration in animals contained on Diabetogenic diet

№	Diabetogenic diet by Kotake Y.	Blood Glucose, mM	
		before	30, 70, 90 days past
1	30 days	4,98±0,22	7,06±0,33
2	70 days	4,98±0,22	6,72±0,25
3	90 days	4,98±0,22	9,27±0,38*

*$p < 0,01$.

Table 2

Concentration of 4,8-dihydroxyquinolin-2-carboxylic acid (4,8DQC) in the Urine

№	Groups of animals	4,8 DQC in the Urine collected for 24h (mcg/ml/24h)	
		before	past
1	Control animals (intacts)	0,045±0,002	0,042±0,003
2	5 days on Diabetogenic diet	0,038±0,003	0,041±0,006
3	93 days on Diabetogenic diet	0,037±0,002	0,335±0,022*

* $p < 0,01$.

State of Histostructure of Endocrine Pancreas tissue

Animals 90th days contained on diabetogenic diet. Endocrine tissue of pancreas: a) fibrinoid changes and formation of a collagenic capsule of islets and thickening of a basal membrane of capillaries (Fig. 1.1, 1.2); b) hydropic dystrophia of B-cells, edematous an endothelium, single capillaries free of erytrocytes and of leukocytes; c) vacuolisation of cytoplasm of B-cells, big hypochromic nuclei (Fig. 1.2); polymorphism of nuclei; d) hydropic changes of nuclei; e)necrosis of central part of β-cells with necrosis of nuclei, disintegration and disappearance of secretory granules; g) lysis of secretory granules on B-cells located on periphery of islets; change of form of cells; h) groups and single β-cells contained in cytoplasm diffuse granularity on the periphery of islets; j) stasis and hyperemia of veins and hemolysis of blood in; k) marked decreasing of insulin in cytoplasm of B-cells; only apical part of B-cells located on perivascular spaces of islets contained reduced amount of deposited insulin.

Thus, after the 90th days containing animals on diet we found marked histological changes in pancreatic islets accompanied by decreasing of insulin content in B-cells.

State of Histostructure of Exocrine Pancreas tissue

Changes on exocrine tissue of pancreas: 1) atrophia of globules of pancreas tissue; atrophied segments are surrounded with wide cavities filled with eosinophilic liquid, connective tissue and fibers (Fig. 1.3);

2) a large amount of collagen fibers in interglobular spaces; free spaces filled with erythrocytes and plasma liquid; 3) edema on space between acinuses; 4) destroying of sinticial structure of apical part, dissociated acinocytes transformed form to oval, decrease of nuclear and cytoplasmatic ratio, the polar structure of cells is changed, more intensive basophilya of cytoplasm; 5)polymorphism of nuclei; discomplectation of apical part of cells, and atrophya of pancreatic cells; 6) formation of niches filling by collagen fibers; 7) necrobiosis of components of epithelial tissue; 8) deformation of nuclei of epithelial tissue cells; necrosis and impregnation of subepithelial layer of internal capsule; 9) periductal sclerosis of some ducts; alteration of epithelial tissue of output ducts (Fig. 1.4); 10) hemorrhagic necrosis of exocrine parenchyma with formation of fibrous tissue; sclerosis of wall of capillaries (Fig.1.5); hyperemia of veins and capillaries; 11) fibrinoid changes of arterio- les, thickening of basal membrane of endothelium; stagnant hyperemia in veins; alterations of endothelial layer of interlobular arteries, proliferation of facile muscle cells; dystrophia and destruction of cells formed blood vessel's wall; 12) capillaries of an arterial link are bloodless, basal membrane of endothelial tissue is thickened; alteration of endothelial cells of interglobular arteries and veins; distrophya and destruction of cell components of most part of wall's vessel; 13) fibrinoid changes of stroma (Fig. 1.11).

State of Histostructure of Liver

State of tissue of Hepar of 90 days of animals contained on diabetogenic diet: 1) dystrophia on periphery and paracentral part of lobules of Hepar tissue with destructive changes of central vein; 2) not correct form of gleam of blood vessels; the wall of the central vessel formed deepening — the funnels pressing in a parenchyma of a hepatic segment, in vein were found granular plasma precipitate and lysis of erythrocytes as stasis of leukocytes nearest vein; infiltration through damaged endothelium (Fig. 1.6) in parenchyma of liver; necrosis of hepatocytes located nearest veins, pycnosis and fragmentation of nuclei; in other hepatocytes: polygonal form, the oval nuclei located in centre of cytoplasm with a large hyperchromic small nucleolus is the centre of nuclei; 3) dystrophia of hepatocytes in combination with appearance in cytoplasm of oval like cells structures; appearance of lypocytes in tissue of liver as symptom of fat infiltration of parenchyma; 4) vacuolar distrophya of hepatocytes especially marked on periphery of segments (Fig. 1.7); 5) hydropic dystrophia of hepatocytes accompanied by desintegration of nuclei and appearance of free of nuclei cells with residual of cytoplasmatic material; 6) disorders of blood circulation in interglobular arteries; destruction and fibrinoid changes of some parts of endothelium; finegrained cell material mixed with large vacuoles in gleam of interglobular vein; the wall of a vein is thinned; 7) interglobular ducts are free of content; decreasing of Glycogen content in hepatocytes (negative SHIFF-histochemical reaction; Table 3); in some segments SHIFF-positive substance in the form of an uneven layer came to light in a wall and in a gleam of the central vein and this part of segment in fat cells were found (Fig. 1.8); 8) in media space the fat infiltration of a parenchyma was observed and partial vacuolar distrophya of cells; the SHIFF-positive substance was found in a wall of capillaries like granules of various color intensity located in cytoplasm of hepatocytes, located between fat cells and cells with vacuolar dystrophia (Fig. 1.9) and destruction of central vein (Fig. 1.10); on periphery — fat cells, vacuolar distrophya and in other segments their central part contained glycogen depot form; in central part of segments the glycogen was found in diffuse disseminated hepatocytes and in the periphery parenchyma showed chromofobe properties that testified to disappearance of depot of glycogen (Fig. 1.6, 1.10); structural signs of protein synthesis in hepatocytes shown as more intensive Pironynophylia of parenchyma tissue; decreasing of RNA content in combination with fat infiltration and vacuolar distrophya of hepatocytes; parts of parenchyma tissue with distrophya of cells accompanied by impairment or complete disappearing of Pyroninophylia; not marked fat infiltration on periphery, vacuolar distrophya of cells in combination with clarification of cytoplasm; weakened Pironinophylia, colliquative necrosis and hydropic degeneration (Fig. 1.12).

Table 3

Glycogen content in Hepatocytes of animals contained on Diabetogenic diet (relative units, r.u.)

№	Groups of animals	Glycogen content in hepatocytes (r.u.)		
		80 days	90 days	120 days
1	Control animals (intacts)	1,94±0,05	1,92±0,07	1,90±0,06
2	80,90 and 120 days on Diabetogenic diet	1,35±0,06	1,36±0,05	1,09±0,03*

* $p < 0{,}01$.

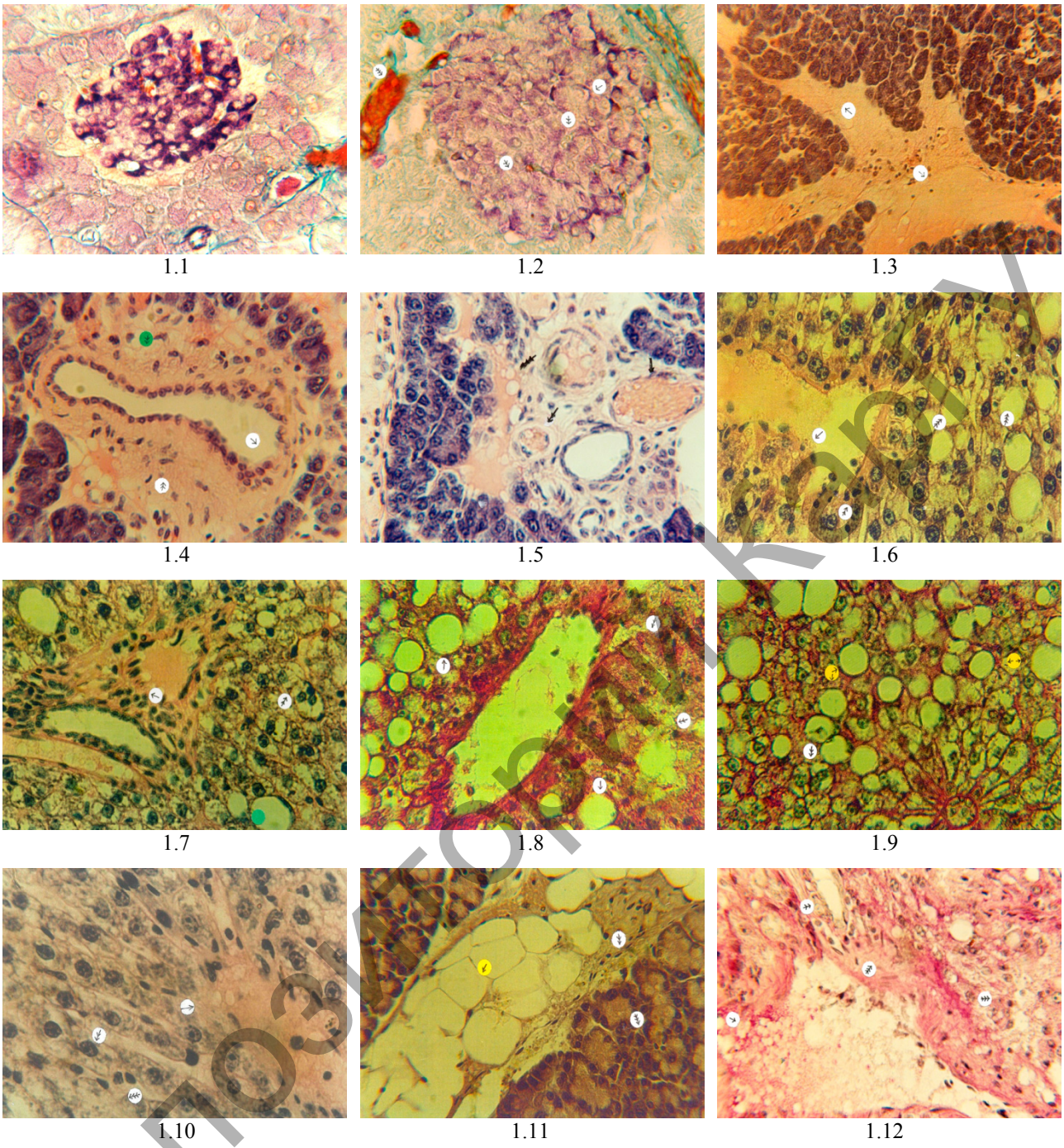

Figure 1

1.1 Intact Pancreatic islet. Histostructure and insulin content in cytoplasm of B-cells without changes. Aldehyde-fucshin, ×280;

1.2 Pancreatic islet. 90 days on diet. Degranulation of B-cells (→), necrosis of B-cells (→→); stasis in vein (→→→). Aldehyde-fucshin, ×280;

1.3 Exocrine tissue of Pancreas. 90 days on diet. Fibrinoid changes of stroma, destruction of acinuses and atrophy of parenchyma (→). Haemalaune-eosin, ×280;

1.4 Exocrine tissue of Pancreas, intralobular ductus; 90 days on diet; destruction of epithelium (→), periductal sclerosis (→→). Haemalaune-eosin, ×280;

1.5 Microcirculation system of Pancreas, 90 days on diet; hyperemia on vein (→), fibrinoid changes of arterioles (→→); plasma infiltration of acinuses (→→→). Haemalaune-eosin, ×280;

1.6 Liver, paracentral part of lobule; 90 days on diet. Destruction of central vein (→), necrosis and leicocytar infiltration (→→); fat infiltration of parenchyma. Haemalaune-eosin, ×280;

1.7 Liver. Peripheral part of lobule and Portal tractus of Liver; 90 days on diet. Destruction and necrosis of arteria (→), leicocytar infiltration; vacuolar distrophya of hepatocytes (→→). Haemalaune-eosin, ×280;

1.8 Liver. Paracentral part of lobule, 90 days on diet. Negative histochemical reacton for Glycogen; fat infiltration of parenchyma (→), necrosis of hepatocytes (→→). Staining by SHIFF-solution, ×280;

1.9 Liver, 120 days on diet.Fat infiltration of parenchyma(→), vacuol distrophya of hepatocytes (→→). Staining by SHIFF-solution, ×280;

1.10 Liver. Destruction of central vein (→), vacuolar distrophya of hepatocytes (→→) and cytolysis; necrosis of cells (→→→). Hemathein-eosin, ×680;

1.11 Exocrine tissue of Pancreas. 30 days on diet. Fat tissue in interglobular space (→), fibrinoid changes of the stroma (→→); discomplectation of acinuses (→→→). Haemalaune-eosin, ×280;

1.12 Liver. Peripheral space of globule and Portal tractus of Liver. Necrosis of interglobular vein (→). Destruction of epithelium of ductus (→→). Hydropic degeneration, colliquative necrosis of hepatocytes (→→→); 90 days on diet. Staining by SHIFF-solution, ×280.

Discussion

Obtained results showed that contrary to experimental diabetes induced by injection of diabetogenic chemicals developed as essential diabetes caused by selective destruction of B-cells only, diabetes induced by diabetogenic metabolites of abnormal Tryptophan metabolism formed in human as result of endogen synthesis, have some important differences. Analogical artificial metabolites (8-oxyquinaldin, 4,8 DQC) result developing of 1 type diabetes in animals past injection of diabetogenic doses of as well as other diabetogenic chemicals as Alloxan, Streptosotozin, Dithizon and derivatives of 8-oxyquinolin. These models not accompanied by developing of primary structure changes outside of pancreatic islets at the same time with destruction of B-cells. Histological changes revealed by us in the Liver and Exocrine tissue of Pancreas of animals with long time developed Xanthurenic diabetes are not belong to direct causes of damage of B-cells but can complicate significantly the developing of diabetes. Liver take part in regulation of Glucose metabolism and stability of blood Glucose concentration due to synthesis and catabolism of Glycogen in hepatocytes. Decreasing of amount of Glycogen in cytoplasm of hepatocytes is as we suppose one of causes which can disturb Glucose metabolism regulation.

The influence of other changes in Liver as marked histological changes: a vacuolar distrophya, necrosis and cytolysis of hepatocytes, hydropic degeneration, colliquative necrosis of hepatocytes, fat infiltration of parenchyma and vascular changes as destruction of central vein, destruction and necrosis of arteria, fibrinoid changes of arteria, now is not investigated yet in details. Now it is not possible to conclude finally what is integrated effect or effect of each of histological changes on developping of diabetes caused by Xanthurenic acid. However it is possible to suppose that integrated effect maybe estimated as not direct factor developing of this model of diabetes but as changes induced by B-cytotoxic chemicals by possible follow ways: aggravation of regulation of Glucose homeostasis as result of disturbances of Glycogen synthesis function, destructive morphological changes in Liver and vascular changes in blood vessels in Exocrine Pancreas tissue as in arteries of Pancreatic islets that formed conditions for disturbances of trophism of Endocrine Pancreas tissue.

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Триптофанның диабетогендік метаболиттерінің себебінен дамитын эксперименталды диабет кезіндегі бауырдың және ұйқыбездің экзокриндік тінінің күйі

Авторлар адамда диабеттің 2-түрінің даму жағдайына жақындатылған эксперименталды диабет кезіндегі бауырдың және ұйқыбездің күйі туралы алғашқы мәліметтерді келтірген. Эксперименталды диабет егде жастағы адамдар ағзасында белсенді түзіліп тұратын 4,8-дигидроксихинолин-2-карбон қышқыл заттың әсерінен дамиды. Диабет пайда болуының тікелей себебі ұйқыбезі В-жасушалардың зақымдануымен қатар, бауырда және ұйқыбездің экзокринді бөлігінде көптеген өзгерістер дамитындығы бірінші рет көрсетілген. Бұл өзгерістер диабет дамуының тікелей себебі болмаса да, оның ағымына едәуір салмақ түсіріп күшейтуі мүмкін.

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Состояние гистоструктуры печени и экзокринной ткани поджелудочной железы при экспериментальном диабете, вызываемом диабетогенными метаболитами триптофана

Авторами приведены первые данные о состоянии печени и экзокринной ткани поджелудочной железы при экспериментальном диабете, приближенном по условиям возникновения и развития к диабету 2 типа у человека и вызываемому 4,8-дигидроксихинолин-2-карбоновой кислотой — веществом, активно синтезирующимся в организме лиц пожилого возраста, в отличие от всех известных сегодня более чем 30 диабетогенных веществ. Впервые было установлено, что одновременно с поражением панкреатических В-клеток, что является непосредственной причиной возникновения диабета, развиваются многочисленные изменения в печени и в экзокринной ткани поджелудочной железы, которые, не являясь прямой причиной развития диабета, могут значительно отягощать и усугублять его течение.