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Concentration of complex Zn^{+2}–chelator around blood vessels in pancreatic islets result alteration and destruction of capillaries

Authors showed that concentration around capillaries wall of complex Zn^{+2}-chelator formed in result of interaction in cytoplasm of B-cells of Zn^{+2}-ions with diabetogenic zincbinding chelators is one of causes of developed vascular changes in capillaries of pancreatic islets. These changes as alteration of basal membrane of capillaries, edema of endothelium, partial destruction of capillaries wall and hyperemia can to aggravate developing of degenerative changes in pancreatic islets induced by Zn^{+2}-chelator that result aggravation developing of diabetes. Meanwhile main cause of developing of diabetes induced by zincbinding chelators is its ability to form toxic complexes with Zn^{+2}-ions in B-cells that result necrosis and destruction of B-cells within short time.

Key words: pancreatic islets, insulin, Zn+2-ions, B-cells, destruction, blood vessel, chelat complexes, vascular changes, diabetogenic derivatives of 8-oxyquinolin.

Background. Diabetogenic derivatives of 8-oxyquinolin (8OX) and Dithizon (DZ) formed in cytoplasm of B-cells toxic for cells chelat complexes with Zn^{+2}-ions. Presence of complex in cytoplasm of B-cells within 15–20 min result necrosis and destructtion of 85–95 % cells [1, 2]. Some of 8OX formed in animals and human as result of disturbances of amino acid metabolism [3].

The complex Zn^{+2}-DZ is formed in B-cells as well visible red granules which located very unevenly in cytoplasm of B-cells [2]. 8-para(toluenesulphonylamino)quinoline (8TSQ), a diabetogenic derivative of 8OX formed in B-cells toxic complex Zn^{+2}–8TSQ as visible green fluorescent granules [2, 9]. Meanwhile it is known that Zn^{+2}-ions in B-cells for med with insulin deposited form of insulin as Zn^{+2}-insulin complex [4] concentrated maximally on apical part of B-cells contacted wall of capillaries of pancreatic islets. It is established also that experimental diabetes caused by derivatives 8-oxyquinolin accompanied by pathological changes of a wall of capillaries [5] developed very quickly — within several days.

Aims of work: 1) to study vascular changes in capillaries of islets in animals with diabetes caused by derivative of 8OX; 2) to investigate indicators of concentration of complex Zn^{+2}-chelator in B-cells located around capillaries in compared with B-cells not contacted capillaries; 3) on the basis of the analysis of results try to answer on question: can be a concentration of toxic Zn^{+2}-chelator complex near capillaries of one of the reasons of development of pathological changes of cages of a wall of capillaries.

Methods. 14 rats, 150–165 g body weight, and 8 rabbits, 2050–2400 g were used. Group 1 (rats): diabetes caused by containing animals 96–104 days on diet induced endogene synthesis of Xanthurenic acid, XA a diabetogenic metabolite of abnormal Tryptophan metabolism (yeast, butter, casein, starch, sugar, salt). Blood glucose control (BG) — weekly; XA in the urine (XAU) — monthly [6]; histology: aldehydefucshine staining method [7]; insulin staining by pseudoisocyanine (PS) [8–10] methods with measuring of intensity of fluorescence; vital staining of Zn^{+2}-DZ complex in B-cells; transmission electron microscopy of ultrasections

of rabbit's pancreas tissue. Group 2 (rabbits): injection of 2 % water-ammonium solution of Dithizon (48,6–50,8 mg/kg); 10 min later dark microscopy of frozen pancreas sections; transmission electron microscopy of rabbit's pancreas tissue 2h past injection of DZ.

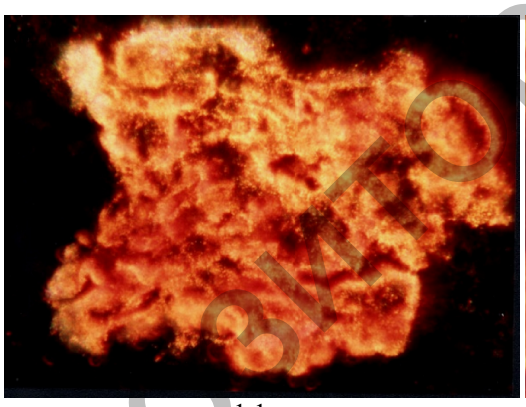
Results. Group 1. Vascular changes in capillaries of islets: thickening of basal membrane in 35±6.4 % of capillaries in 40±5.8 % of islets, edema of endothelium in 26±5.9 % capillaries in 42±6.2 % of islets. Blood glucose concentration (BG): before — 4.4±0.5 mM; 96^{th}-104^{th} day — 9.8±2.4 mM; XAU before — 0,032±0,004 mcg/ml, 93–98 days later — XAU — 0,377±0,039 mcg/ml; insulin content (AB): IG — 1.34±0.05 (control: 1.95±0.08); PS — 1.32 ±0.04 (control: 2.02±0.06). Histology of pancreas: hydropic degeneration, vacuolization of cytoplasm, necrosis and death of B-cells, lysis of B-granules, hydropic changes of nuclei.

Group 2. A large amount of red granules of complex DZ-Zn^{+2}-ions concentrated in B-cells of rabbit pancreas (A/B index — 6.76±0.62; A — contacted with capillaries, B — concentration of granules in cells not contacted with capillaries, fig.1.1–1.4) and 3.31±0.29 in B-cells of mice islets (fig. 1.5–1.7). Concentration of Zn^{+2}-ions correspond to concentration of complex insulin-Zn^{+2}-ions in B-cells (fig. 1.8–1.10, table). Results showed that concentration as of Zn^{+2}-ions as insulin in cytoplasm of B-cells is 6 times more in B-cells located around capillaries in islets of rabbits and 3 times in mice comparatively with other parts of cytoplasm of cells. Results of electron microscopy investigation: destruction of cell's matrix on 84–95 % of the surface of B-cells in all investigated islets (fig. 1.11, 1.12).

Table

Concentration of Zn^{+2}-DZ and Zn^{+2}-insulin complexes in B-cells

№	Animals	Concentration of granules of Zn^{+2}-DZ in cytoplasm of B-cells (A/B index)	Concentration of Zn^{+2}-insulin complex in cytoplasm of B-cells (A/B index)	p
1	Rabbits	●6.76±0.62*	●5.88±0.54	●>0.05
2	Rats	●3.31±0.19*	●3.10±0.22	*<0.005



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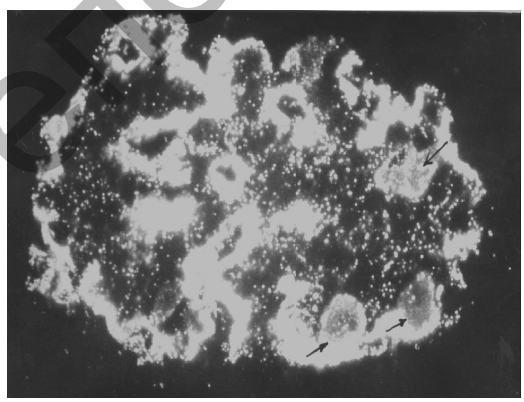

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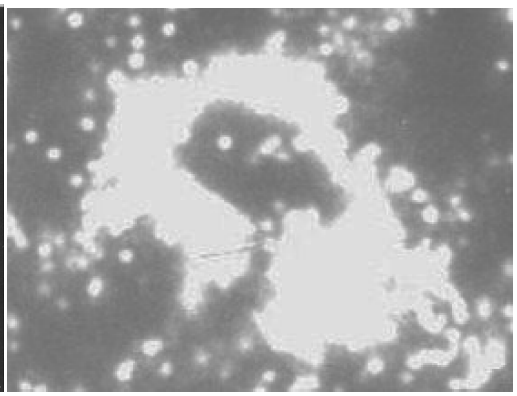

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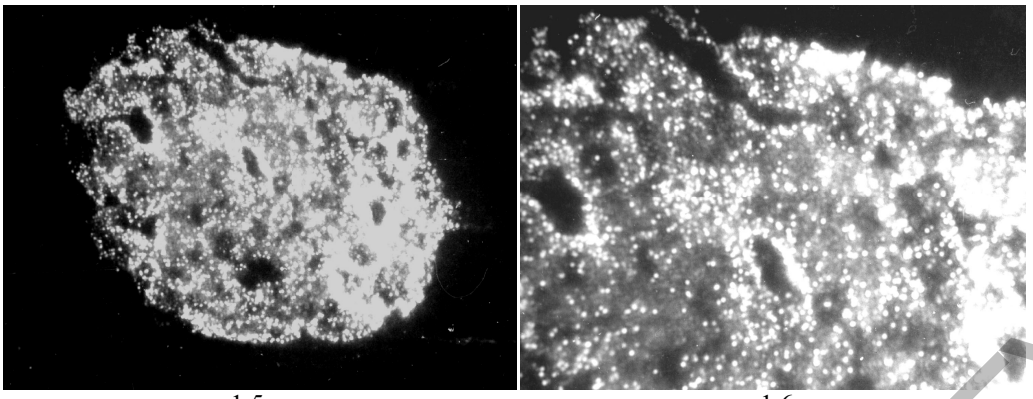
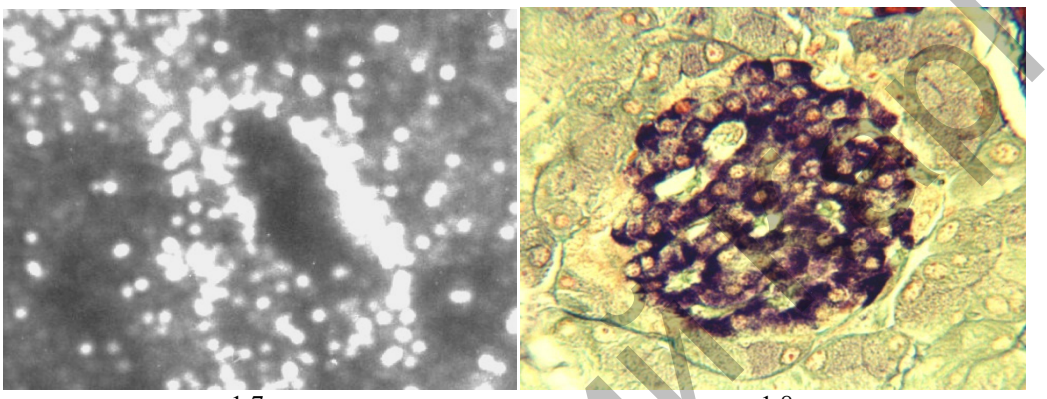
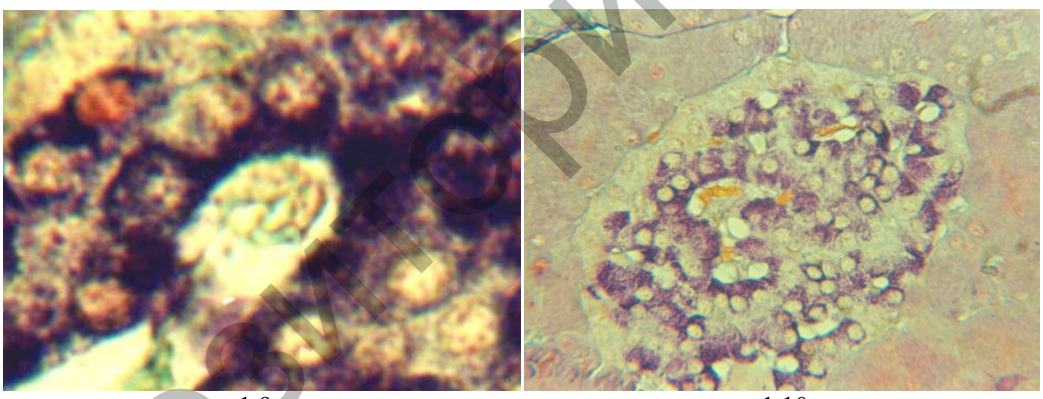


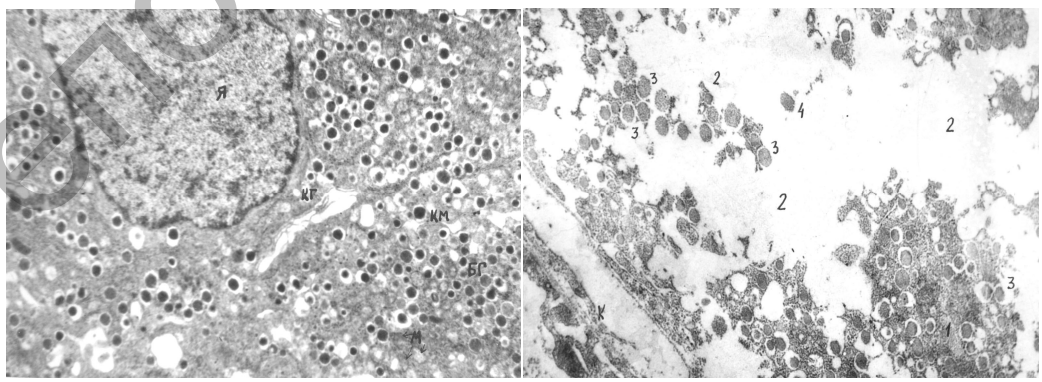
1.5 1.6



1.7 1.8



1.9 1.10



1.11 1.12

1.1 Islet of rabbit. Dithizon 49.6 mg/kg; frozen section 7 mcm; darc microscopy: maximal concentration of red complex DZ-Zn^{+2} round capillare (central part); ×280;

1.2 Islet of rabbit. (fragment of 1.1). Dithizon 49.6 mg/kg; frozen section 7 mcm; darc microscopy: maximal concentration of red complex DZ-Zn^{+2} round capillare; ×680;

1.3–1.4 Islet of rabbit. Dithizon 49.6 mg/kg; frozen section 4 mcm; darc microscopy: maximal concentration of complex DZ-Zn^{+2} around capillaries, ×280; ×930;
1.5–1.7 Islet of white mice. Dithizon 46.2mg//kg; frozen section 4 mcm; darc microscopy: maximal concentration of complex DZ-Zn^{+2} around capillaries, ×280; ×540; ×920;
1.8–1.9 Islet of intact rat. Aldehydefucshine. Maximal concentration of insulin-Zn^{+2}-complex (violet color) around capillaries, ×280; ×610;
1.10 Islet of diabetic rat. Aldehydefucshine. Maximal concentration of reduced amount of insulin-Zn^{+2}-complex around capillaries, ×280;
1.11 Intact islet of rabbit. Transmission electron microscopy: cell's matrix and ultrastructures without changes; ×3650;
1.12 Islet of rabbit 2h later injection of Dithizon: total destruction of cell's matrix and of B-granules; ×4100. Preparats and microphotos 1.3–1.7, 1.11, 1.12: by Prof. G.G.Meyramov; 1.1, 1.2, 1.8–1.10 by Prof. A.A.Kikimbaeva, Prof. Kohnert K.-D., Prof. G.G.Meyramov, 2008–2013.

Figure 1

Discussion. It is known that main cause of destruction of diabetes induced by chelat active chemicals is ability of drug to form toxic complexes with Zn-ions that result destruction of B-cells within a few minutes. Some of these chemicals formed in animals and human as result of metabolic disturbances and some of its — derivatives of oxyquinolin and 8-oxyquinolin — contain as component of drugs as Isoniazide, Ketotyphenum, Enteroseptol, Salmeterol, Chalcogen, Intestopanum, Mcxase, Mexaform, Colposertine, Chiniofonum, Chinosolum, Chlorchinaldolum, 5-NOK, Enterusan, Vioform, Intetrix, Dermosolon, Chinofucinum. Later it was established that forming of toxic chelat with Zn^{+2}-ions contained in prostate of animals accompanied by developing of destruction of cells of gland. Meanwhile pancreas tissue is more sensitive for destructive action of chelat complexes. In previous decades of experiences we paid attention that maximal number of granules of chelats complexes are concentrated around blood capillaries in pancreatic islets [11]. This fact was confirmed visually using Dithizone histochemical technic as aldehyde-fucshine and pseudoisocyanine histochemical methods staining of complexes DZ-Zn^{+2}-ions and of Insulin. This same time in we found using high specific histochemical fluorescent method revealing of Zn^{+2}-ions in B-cells that maximal amount of ions are concentrated in B-cells located exactly around blood capillaries [12]. There are questions: 1) whether high concentration of chelat complexes located around capillaries to provoke alteration of capillaries?; 2) whether developed in pancreatic islets a vascular changes to aggravate blood circulation in islets? Our results showed that contrary to vascular changes developed as latest complication of diabetes, in our experiences they developed evidently more rapidly — within 1–2 weeks. On the base of obtained results we suppose that: 1) vascular morphological changes developed in pancreatic islets may be determined by alteration caused by high concentration of complex «Zn^{+2}-chelator» around capillaries; 2) vascular changes possess not directly to aggravate developing of diabetes caused by diabetogenic derivatives of 8OX and by Dithizon.

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Қылтамырлар қабырғасының бұзылуымен қоса жүретін панкреатиттік аралшықтардағы қылтамырлар маңайындағы «Zn^{+2}–хелатор» кешенінің қалыптасуы

Авторлар химиялық кешен түзетін заттармен тудыратын диабет кезіндегі панкреатиттік аралшықтардың қылтамырлары қабырғаларының тамырлық өзгерістерінің негізгі себебі В-жасушаларында Zn^{+2}-хелатор кешенін түзетін қылтамырлар маңайындағы жоғары концентрация болып табылады деп белгілеген. Бұл тамырлық өзгерістер В-жасушаларды бұзудың алғашқы себебі болмай, қанмен толығуының әлсіреуінің нәтижесінде диабет барысын ұлғайтатын аралшықтардағы дегенеративтік өзгерістердің дамуын жүзеге асыратын екіншілік себебі болады.

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Формирование комплекса «Zn^{+2}–хелатор» вокруг капилляров в панкреатических островках, сопровождающееся деструкцией их стенки

Авторами показано, что, вероятно, основной причиной сосудистых изменений стенки капилляров панкреатических островков при диабете, вызываемом химическими комплексообразующими веществами, является высокая концентрация вокруг капилляров образующегося в В-клетках комплекса Zn^{+2}–хелатор. Развивающиеся сосудистые изменения, не являясь первичной причиной разрушения В-клеток, способны вторично, в результате ухудшения кровоснабжения, способствовать развитию дегенеративных изменений в островках, усугубляющих течение диабета.

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