



Histochemical Investigation of Influence of Diabetogenic Zinc Binding Chemicals *In Vitro* on Human Pancreatic β -Cells and its Prevention

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Abstract

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BACKGROUND: Amount of zinc-ions is correspond to insulin content in cytoplasm of β -cells. Diabetogenic zinc binding chemicals (DZC) formed with zinc in β -cells chelat complex that result destruction and death of β -cells within 10–15 min in animals.

AIM: The aim of the study was to investigate using of specific histochemical methods the content of zinc and insulin in β -cells of pancreatic islets of human intact fetal pancreas tissue and under action of diabetogenic and non-diabetogenic zinc-binding substances diabetogenic zinc diethyldithiocarbamate (DZ and DDC).

METHODS: Pancreas from 8-week-old human embryos and adult rabbits were used. Fixed or frozen islets were used *in vitro* effect. Histochemical methods for insulin and zinc in β -cells were used.

RESULTS: Results showed that zinc in human β -cells is clearly revealed using of histochemical methods. In embryo pancreas, not integral islets were found as clusters of cells or even individual β -cells. Insulin content in β -cells is for 7–20% lower and Zn^{2+} by 16–18% in compared with rabbits; Zinc in human β -cells formed chelat complexes with 8PTSQ and DZ as Zn^{2+} -8PTSQ and Zn^{2+} -DZ. Sodium DDC, a non-diabetogenic Zn^{2+} chelator, formed not toxic complexes with Zn^{2+} in fetal β -cells that protect cells of destruction caused by DZC as in animal's pancreas.

CONCLUSION: (1) Intracellular reactive zinc contained in human fetal pancreatic β -cells clearly revealed using of histochemical methods and also (2) demonstrated that DZC in human β -cells result formation with zinc of chelat complexes. (3) As in animals, non-toxic chemical as DDC is able to block zinc in β -cells that result prevention of its destruction caused by DZC.

Introduction

Zinc is involved in multiple processes within the exocrine and endocrine pancreas. Pancreatic β -cells accumulate Zn^{2+} in secretory granules where it is provided for processing of storage and secretion of insulin [1], [2]. Several studies have suggested that Zn^{2+} plays an important role in the pathology of diabetes, that is, Zn^{2+} deficiency causes hyperglycemia and hyperinsulinemia. Among about 19 diabetogenic chemicals [3], [4], [5], capable for selective destroying β -cells, as 18 belong to Zn^{2+} -binding derivatives of 8-oxyquinoline diabetogenic zinc binding chemicals (DZC). Affinity of these substances for Zn^{2+} is high. Injection of DZC caused formation in β -cells of toxic chelat complexes as Zn^{2+} -DZC that result destruction of β -cells within short time and development of first type diabetes in many types of animals. Regarding human fetal pancreas, there are not investigations demonstrate the presence of Zn^{2+} in β -cells as of possible its interaction

with DZC. Meanwhile there are some antimicrobial drugs that contain derivatives of 8-oxyquinolin as active antimicrobial component in the structure.

In view of the importance of zinc for the β -cell function, it was the goal of the present work to investigate does interacted Zn^{2+} of fetal human pancreatic islet's β -cells with DZC and, if yes, how deleterious interactions with chelators might be prevented for to protect β -cells of destroying.

Materials and Methods

Animals

Pancreas of rabbits and human fetal were used.

Reagents

8-Para (toluenesulphonamidoquinoline (8PTSQ) from Institute of High Pure Reagents (Moskva, Russia); Collagenase from Boehringer Mannheim GmbH (Germany), Victoria 4R (dimethylnaphtylmetan) from MERCK (Germany) and from FERAK Berlin (Germany), Diethylpseudoisocyanine chloride from SERVA Finebiochemica (Germany), aldehyde-fuchshine from Avocado Chemical Company (USA) and reagents from DAKO (Denmark) for insulin immunohistochemistry.

Human and Rabbit pancreatic tissue

Three human fetal pancreas 8 weeks of gestational age were obtained from the Department of Obstetrics as result of interrupting of pregnancy due to medical causes. Pancreas from 12 adult rabbits were used as control. One portion of each pancreas was fixed in Bouin's and other portions were treated with collagenase [6] for isolation of islets for investigate direct action of DZC on β -cells. About 2% collagenase solution containing buffer pH 7.33–7.40 was used 3 times approximately 1 min. each. After rinsing in Hanks islets were separated in Dextran solution, and incubated for 3 h in Medium 199 + 5.6 mM glucose containing bovine serum albumin.

Histochemistry and histofluorimetry

Fixed tissue paraffin 4–5 μ m sections of pancreas were used for histochemistry of insulin and Zinc in β -cells using of methods as aldehyde fuchshine [7], Diethylpseudoisocyanine chloride [8], [9], [10], [11], [12], [13], [14], [15] Victoria Blue 4R [9], or with anti-insulin antibody using the indirect immunoperoxidase method [10]. The fluorochrome 8-para (toluenesulphonylamino) quinoline (8PTSQ) as well as Dithizone for staining and to analyze Zn^{2+} distribution in the pancreatic islet's sections were used [13]. The content of insulin and of Zn^{2+} deposited in the β -cells was estimated by histofluorimetry according to the intensity of fluorescence of the Zn^{2+} –8PTSQ complex and of density of the Zn^{2+} –dithizone complex [11]. Intensity of fluorescence (IF) measured using of photometry of β -cells using of histofluorimetric complex [11]. Calculation of the parameter K (insulin or zinc content in β -cells calculated in relative units, ru) was performed as $K = IF1/IF2$, where IF1 is the IF of the human intact β -cells; IF2 is the IF of β -cells destroyed by diabetogenic zinc (DZ) set as 1.00. Calculation of parameter K for staining with dithizone and quantification using light microscopy was done according to the formula $K = AB1/AB2$, where AB1 is the light absorbance of intact β -cells; AB2 the light absorbance of treated by chelators β -cells. The inverse relationship gives the light absorption level, that is, staining density.

For staining of Zn^{2+} in β -cells, frozen sections 4–5 μ m were used. Frozen sections were incubated for about 10 s with 0.04% acetone solution of 8PTSQ in

and investigated using fluorescent microscope. Staining by Dithizone: Sections were incubated in ammonium-buffered water solution contains 2% DZ for 20–25 s for staining the Zn^{2+} -ions. After rinsing in distilled water, sections were investigated using dark microscopy. Next, 2–3 drops of 2% solution of the sodium salt of DKK were layered over the sections for about 15–20 s at room temperature followed subsequently by 2–3 drops of DZ solution, which remained for another 20–30 s on the sections. Thereafter, section were carefully rinsed in distilled water and investigated.

Statistic analysis

t-criterion of student was used for calculation of $M \pm m$.

Results

Three main types of islets have been identified in fetal human pancreas (Table 1). In contrast to rabbit's pancreas, β -cells in sections of human fetal pancreas were organized either in aggregates consisting of 30–40 (Table 1, Figure 1a, d and e), 20–30 cells (Table 1, Figure 1b and f), and 5–10 cells (Table 1 and Figure 1c) or appeared as single β -cells distributed through the pancreas. The number of compact islets β -cell aggregates per square of the pancreas sections; tissue was almost 2, 5–3 times lower in human fetal pancreas than in the rabbit's pancreas. Large and more compact islets (30–40 β -cells) are the dominant islet type in human fetal pancreas. Figure 1 displays the staining of insulin and zinc sections of pancreas and of isolated islets. This is demonstrated in Table 2 where the different staining results are shown in compared with neonatal rat pancreas. Histochemical detection by staining of islets with 8PTSQ and DZ revealed that the insulin and Zn^{2+} content in fetal human islets was, respectively, for 8, 1–20% lower than in rabbit's islets depending of methods of staining (Table 2): 1.54 ± 0.07 r.u.; 1.61 ± 0.04 ; 1.76 ± 0.04 ; and 1.59 ± 0.09 in compared with 1.84 ± 0.08 r.u.; 2.02 ± 0.03 ; 1.91 ± 0.05 ; and 1.85 ± 0.12 in rabbit's islets. Formation of Zn^{2+} –DZ complex in human β -cells accompanied by marked decreasing of insulin Zn^{2+} content as of destruction of majority β -cells: 1.08 ± 0.03 r.u.; 1.04 ± 0.02 ; 1.12 ± 0.05 ; and 1.14 ± 0.04 in compared with 1.51 ± 0.06 ; 1.58 ± 0.03 ;

Table 1: Cytoarchitecture of β -cells in sections of fetal human and Rabbit pancreas (%)

Distribution of islet β -cell types (%)				
Pancreas	Large and compact islets with numerous β -cells	Group 1 Aggregates of 30–40 β -cells	Group 2 Aggregates of 25–30 β -cells	Group 3 Aggregates of 5–10 β -cells
Rabbit islets (n = 31)	$87.0 \pm 7.8\%$	$6.0 \pm 1.5\%$	$4.0 \pm 0.5\%$	$3.0 \pm 0.5\%$
Human islets	-	$34.0 \pm 6.5\%$	$18.0 \pm 2.3\%$	$48.0 \pm 6.8\%$

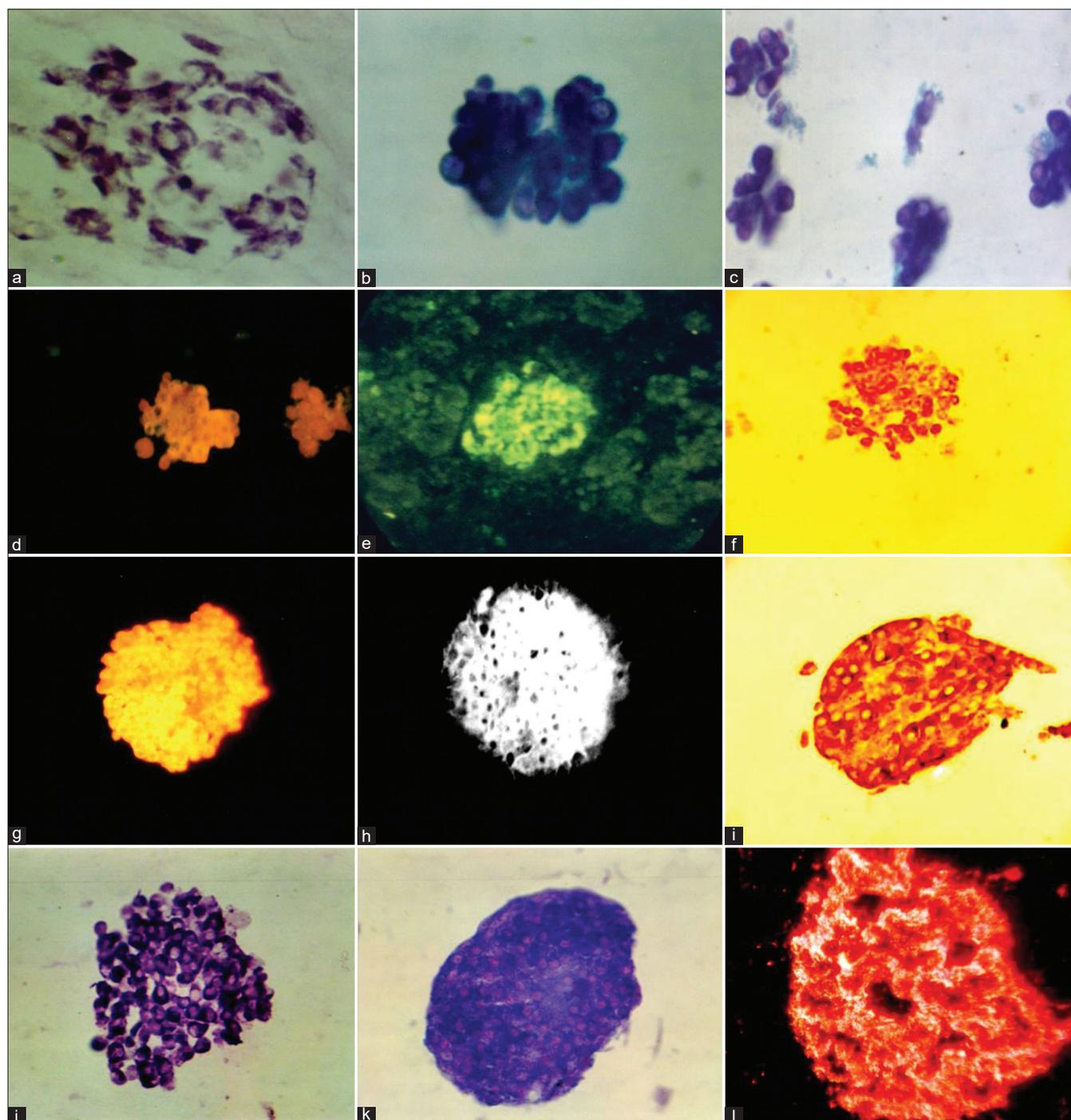


Figure 1: Microphotographs of pancreatic islets and tissue sections stained for insulin and zinc-ions from fetal human (a-f), rabbit (g-l). Pancreas section stained with aldehyde-fuchsin showing aggregated insulin-positive cells, magnification $\times 280$ (a). Group of densely aggregated β -cells stained with Victoria blue 4R, $\times 400$ (b). Scattered groups of insulin-positive cells stained with aldehyde-fuchsin, $\times 400$ (c). Staining with pseudoisocyanine of a group of aggregated islets show red fluorescence for insulin, $\times 120$ (d) Demonstration of Zn^{2+} in β -cells as detected by intensive green fluorescence, $\times 120$ (e). Positive immunostaining for insulin observed in aggregated β -cells, $\times 120$ (f). Pseudoisocyanine staining indicates the presence of insulin-containing β -cells in isolated rabbit's islet, $\times 120$ (g). The existence of numerous Zn^{2+} -containing cells in rabbit's islet as indicated by intensive green fluorescence, $\times 120$ (h). Immunoperoxidase staining for insulin shows the presence of abundant β -cells in a rabbit's islet, $\times 300$ (i). Aldehyde-fuchsin staining for insulin contained in β -cells as indicated by the violet color, $\times 280$ (j). Staining with Victoria blue 4R of rabbit's islet demonstrates the presence of numerous insulin-positive (blue) cells, $\times 160$ (k). Dithizone staining reveals the existence of large amounts of Zn^{2+} (red-orange) in rabbit's islet, $\times 280$ (l)

1.74 ± 0.12 ; and 1.57 ± 0.04 after preventive action of diethyldithiocarbamate (DDC). Negative reaction for Zn^{2+} after action of DDC determined by protective not diabetogenic binding of Zn^{2+} with DDC: 1.04 ± 0.03 and 1.06 ± 0.04 r.u. in compared with 1.67 ± 0.05 and 1.62 ± 0.11 r.u. in intact β -cells.

Discussion

It is known the role of Zn on pancreatic β -cell function, including insulin synthesis and secretion, Zn signaling in the pancreatic islet, the redox functions of Zn,

Table 2: Insulin and Zn^{2+} content in fetal human and Rabbit pancreatic islets before and after interaction with diabetogenic (DZ) and non-diabetogenic (DDC) Zn^{2+} -chelators (r.u.-relative units)

Pancreas	Histochemical staining methods					
	Insulin				Zn^{2+}	
	Aldehyde-fuchsin	Pseudoisocyanine	Victoria-4R	Immunostaining	8PTSQ	Dithizone
1 Rabbit's β -cells	1.84 \pm 0.08 (n = 20)	2. \pm 0.03 (n = 20)	1.91 \pm 0.05 (n = 20)	1.85 \pm 0.12 (n = 16)	2.04 \pm 0.07 (n = 18)	1.93 \pm 0.12 (n = 12)
2 Fetal human β -cells	1.54 \pm 0.07 (n = 12)	1.61 \pm 0.04 (n = 10)	1.76 \pm 0.04 (n = 12)	1.59 \pm 0.09 (n = 10)	1.67 \pm 0.05 (n = 14)	1.62 \pm 0.11 (n = 12)
3 Fetal human β -cells+DZ	1.08 \pm 0.03* (n = 11); destruction of β -cells	1.04 \pm 0.02* (n = 10); destruction of β -cells	1.12 \pm 0.05* (n = 12); destruction of β -cells	1.14 \pm 0.04* (n = 10); destruction of β -cells	1.03 \pm 0.01* (n = 9); absence of Zn	1.07 \pm 0.06* (n = 12); absence of Zn
4 Fetal human β -cells+DDC+DZ	1.51 \pm 0.06 (n = 8)	1.58 \pm 0.03 (n = 10)	1.74 \pm 0.12 (n = 12)	1.57 \pm 0.04 (n = 10)	1.04 \pm 0.03 (n = 11); binding of Zn with DDC	1.06 \pm 0.04 (n = 11); binding of Zn with DDC
5 Rabbit's β -cells+DZ	1.06 \pm 0.04* (n = 11); destruction of β -cells	1.03 \pm 0.02* (n = 11); destruction of β -cells	1.15 \pm 0.08 (n = 12); destruction of β -cells	1.16 \pm 0.07* (n = 10); destruction of β -cells	1.02 \pm 0.01* (n = 9) absence of Zn	1.11 \pm 0.06* (n = 12); absence of Zn
6 Rabbit's β -cells+DDC+DZ	1.82 \pm 0.03 (n = 11);	1.98 \pm 0.07 (n = 11);	1.86 \pm 0.11 (n = 12);	1.79 \pm 0.05 (n = 10);	1.04 \pm 0.01* (n = 9) binding of Zn with DDC	1.09 \pm 0.05* (n = 12); binding of Zn with DDC

n: number of sections; * p < 0.005 compared with Groups 1 and 2. DZ: Diabetogenic zinc, DDC: Diethylthiocarbamate.

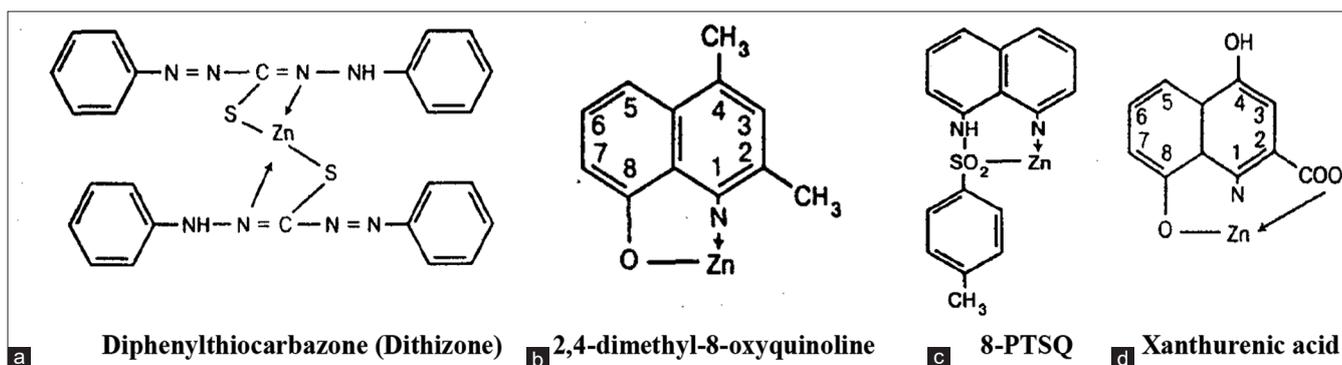


Figure 2: Chemical structure of chelat complexes formed on reaction with diabetogenic Zn^{2+} -binding chemicals [5]. (a) Diphenylthiocarbazon (dithizone), (b) 2, 4-dimethyl-8-oxyquinoline, (c) 8-PTSQ, (d) xanthurenic acid

and its target genes [16]. Using of 8PTSQ and Dithizone methods for staining of Zn, it was demonstrated the presence of marked amounts of Zn^{2+} in islets of human fetal pancreas. It was showed the presence of marked amount of deposited insulin in β -cells using of specific for insulin methods of staining of insulin. The reduced amount of zinc and insulin in the β -cells of the human embryo can be explained by the early period of embryonic development.

Previously, when studying in experiments on animals, the diabetogenic properties of DZC were convincingly proved that the formation of a complex Zinc-helator (DZC) in the β -cells of rabbits, horses, dogs, cats, and hamsters leads in 95–100% result destruction of β -cells and development of Type 1 diabetes. It was found that any causes that prevented the formation of the complex in the β -cells protect the development of diabetes in all experimental animals (Lazaris Y.A., Meyramov G.G., Bavel'sky Z.E., Korchin V.I. (1964–1977). It was confirmed that can be two ways of such reasons: (a) Preliminary blocking of zinc in β -cells with non-non diabetogenic zinc chelators as derivatives of Dithiocarbamic acid and amino acids Cystein and Gluthatione (Bavel'sky Z.E., Meyramov G.G., Shaybek A.Z., 1989–2020) and (b) the preliminary elimination of zinc from the B-cells before the action of influence of DZC. In both cases the formation of a toxic complex of zinc-DZC became impossible and diabetes in animals did not develop. This was confirmed in the experiments *in vitro* (1987–2020).

It was logical to expect the question that would inevitably arise before us: Can a zinc contained

in human β -cells to form toxic complexes with diabetogenic chelators? And if such a complex is formed, is it possible to prevent its formation by binding with the non-diabetogenic chelator as DDC?

The results showed that diabetogenic derivatives of 8-oxyquinoline and of DZ contrary to sodium DDC formed Zn^{2+} -chelate complexes in the β -cells of fetal human pancreas as in β -cells of rabbit pancreas. It has previously reported that these complexes are most stable if Zn^{2+} is located between N, S, or O atoms of the chelating agent. As an example, Figure 2 displays the disposition of atom of Zn^{2+} in molecule of chelat complex. It is confirmed that this complex formation results destruction of majority of β -cells and development of diabetes [5], [12], [13]. Isomers of 8-oxyquinoline, which do not contains such a ring-structure in position 8 of molecule of chelator with an active binding center, are not capable of forming complexes with Zn^{2+} ions in β -cells and possess no diabetogenic properties [1], [5].

It was showed that human β -cells are sensitive as β -cells of all investigated previously animals to cytotoxic chemicals, it is possible that 8-oxyquinoline derivatives potentially are able of disrupting β -cell function by formation of toxic Zn^{2+} -complexes.

The results of this study have made clear that intracellular reactive zinc contains in human fetal pancreatic β -cells as early as 8-weeks at gestation. The results showed that zinc in human β -cells clearly is revealed using of various histochemical methods as in

animals formation in β -cells of toxic complexes with zinc, which induce damage and destruction of β -cells. As in animals non-toxic chemical as DDC is able to block zinc in β -cells that prevent its destruction caused by DZC.

Conclusions

1. Results of this study have made clear that intracellular reactive zinc and insulin are revealed using of specific for zinc and insulin histochemical methods in human fetal pancreatic β -cells as early as 8-weeks at gestation;
2. Zinc of human β -cells formed with Dithizone and 8PTSQ chelat complexes as in β -cells of rabbits;
3. Non-toxic chelator as DDC possess Zn^{2+} -chelating properties is able to prevent formation of chelat complexes of Zn^{2+} with DZC in human β -cells and protect β -cells of destroying;
4. Formation of Zinc-chelator complex in human β -cells can result of destruction and death of human β -cells as in all sorts of investigated animals that always result development of 1 Type of experimental diabetes which can be prevented by DDC.

Disclosure Statement

All authors declare no potential financial interest or any commercial association that might present a potential conflict of interest. All authors declare that no competing financial interests exist.

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