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

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Abstract

BACKGROUND: Amount of zinc-ions is correspond to insulin content in cytoplasm of E-cells. Diabetogenic zinc binding chemicals (DZC) formed with zinc in E-cells chelat complex that result destruction and death of E-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 E-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 E-cells were used.

RESULTS: Results showed that zinc in human E-cells is clearly revealed using of histochemical methods. In embryo pancreas, not integral islets were found as clusters of cells or even individual E-cells. Insulin content in β-cells is for 7–20% lower and zinc in human ß-cells formed chelat complexes. (3) As in animals, non-toxic chemical as DDC is able to block zinc in ß-cells that result prevention of its 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 DCC 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 Zn2+ in secretory granules where it is provided for processing of storage and secretion of insulin [1, 2]. Several studies have suggested that Zn2+ plays an important role in the pathology of diabetes, that is, Zn2+ deficiency causes hyperglycemia and hyperinsulinemia. Among about 19 diabetogenic chemicals [3], [4], [5], capable for selective destroying β-cells, as 18 belong to Zn2+-binding derivatives of 8-oxyquinoline diabetogenic zinc binding chemicals (DZC). Affinity of these substances for Zn2+ is high. Injection of DZC caused formation in β-cells of toxic chelat complexes as Zn2+-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 Zn2+ 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 Zn2+ 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, Rus- sia); Collagenase from Boehringer Mannheim GmbH (Germany), Victoria 4R (dimethylaminopyrrolmetan) from MERCK (Germany) and from FERAK Berlin (Germany), Diethylpseusoisocyanine chloride from SERVA Finebiochemica (Germany), aldehyde-fuchsin 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 Obst-etrics 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.

Histochecmistry and histofluorimetry

Fixed tissue paraffin 4–5 Pm sections of pancreas were used for histochecmistry of insulin and Zinc in β-cells using of methods as aldehyde fuchsin [7], Diethylpseusoisocyanine chloride [8], [9], [10], [11], [12], [13], [14], [15] Victoria Blue 4R [9], or with anti-insulin antibody using the indirect immunoperoxida- se method [10]. The fluorochrome 8-para (toluenesulphonlamino) quinoline (8PTSQ) as well as Dithizone for staining and to analyze Zn²⁺-distribution in the pancreatic islet’s sections were used [13]. The content of insulin and of Zn²⁺ deposited in the β-cells was estimated by histofluorimetry according to the intensity of fluorescence of the Zn²⁺–8PTSQ complex and of density of the Zn²⁺–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 Zn²⁺ in β-cells, frozen sections 4–5 Pm 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 Zn²⁺-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 ± 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 throughout 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 Zn²⁺ 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 Zn²⁺–DZ complex in human β-cells accompanied by marked decreasing of insulin Zn²⁺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 (%)

<table>
<thead>
<tr>
<th>Distribution of islet β-cell types (%)</th>
<th>Pancreas</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aggregate of larger and compact β-cells</td>
<td>30–40</td>
<td>8.6 ± 1.5%</td>
<td>4.0 ± 0.5%</td>
<td>3.0 ± 0.5%</td>
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<tr>
<td>Aggregate of 25–30 β-cells</td>
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<td>Aggregate of 5–10 β-cells</td>
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Rabbit islets (n = 31)

| Human islets | 34.0 ± 6.5% | 18.0 ± 2.3% | 48.0 ± 6.8% |

1.74 ± 0.12; and 1.57 ± 0.04 after preventive action of diethyldithiocarbamate (DDC). Negative reaction for Zn\textsuperscript{2+} after action of DDC determined by protective not diabetogenic binding of Zn\textsuperscript{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 ß-cells.

**Discussion**

It is known the role of Zn on pancreatic E-cell function, including insulin synthesis and secretion, Zn signaling in the pancreatic islet, the redox functions of Zn,
and its target genes [16]. Using of 8PTSQ and Dithizone methods for staining of Zn, it was demonstrated the presence of marked amounts of Zn²⁺ 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 Zn²⁺-helator (DZC) became impossible and diabetes in all experimental animals (Lazaris Y.A., Meyramov G.G., Babelsky Z.E., Shaybeyk A.Z., 1989–2020) and (b) the preliminary elimination of zinc from the β-cells before the action of cytotoxic chemicals, it is possible that 8-oxyquinoline isomers of 8-oxyquinoline, which do not contain such a ring-structure in position 8 of molecule of chelator with sodium DDC formed Zn²⁺–complexes. Isomers of 8-oxyquinoline and DZ contrary to sodium DDC formed Zn²⁺–chelat complexes in β-cells of fetal human pancreas as in β-cells of rabbit pancreas. It has previously reported that these complexes are most stable if Zn²⁺ is located between N, S, or O atoms of the chelating agent. As an example, Figure 2 displays the disposition of atom of Zn²⁺ 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]. 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 Zn²⁺–complexes.

The results showed that diabetogenic derivatives of 8-oxyquinoline and DZ contrary to sodium DDC formed Zn²⁺–chelat complexes in β-cells of fetal human pancreas as in β-cells of rabbit pancreas. It has previously reported that these complexes are most stable if Zn²⁺ is located between N, S, or O atoms of the chelating agent. As an example, Figure 2 displays the disposition of atom of Zn²⁺ 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]. 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 Zn²⁺–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 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 Zn²⁺–chelat complexes in β-cells of fetal human pancreas as in β-cells of rabbit pancreas. It has previously reported that these complexes are most stable if Zn²⁺ is located between N, S, or O atoms of the chelating agent. As an example, Figure 2 displays the disposition of atom of Zn²⁺ 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]. 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 Zn²⁺–complexes.

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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 Zn²⁺ chelating properties is able to prevent formation of chelat complexes of Zn²⁺ 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.

References