Interaction of diabetogenic chelat active chemicals with Zn\(^{+2}\)-ions in pancreatic B-cells and its role for developing of death of pancreatic B-cells

Authors showed that interaction of diabetogenic chelators as Diphenylthiocarbazone and formed in Human some derivatives of 8-oxyquinolin with Zn\(^{+2}\)-ions contained in B-cells result destruction of cells and developing of 1 type diabetes. Preliminary elimination of Zn\(^{+2}\) — ion- from cells or blocking ions by not diabetogenic chelators protect B-cells of interaction with diabetogenic substances and as result — of developing of diabetes.

Keywords: Diabetes, Diphenylthiocarbazone (Dithizon), Zn\(^{+2}\), B-cells, fluorescence, Human, Rabbits, Dogs, Mice, Rats, Kats, Histochemical results, Revealing, Destruction.

**Background.** It is known that diabetogenic derivatives of 8-aren(sulphonylamino)quinolin as 8-metan(sulphonylamino)quinolin \[8MSQ\], 8-bensol(sulphonylamino)quinolin \[8BSQ\], 8-para(toluenesulphonyl-amino)quinolin \[8PTSQ\], 4,8-(dihydroxyquinolin)-2-carboxylic acid (Xanturenic Acid, XA) as diphenylthiocarbazone (Dithizon) \[DZ\] possess high chemical affinity for Zn\(^{+2}\)-ions and in vitro formed color complexes as \(\text{Zn}\(^{+2}\)-chelator}\[1, 2\]. 8MSQ and 8TSQ formed fluorescent yellow and green complexes with Zn\(^{+2}\)-ions visible using fluorescent microscopy and Dithizone formed red DZ-Zn\(^{+2}\)-ions complex visible using dark microscopy. Maximum of absorbance of Zn\(^{+2}\)-DZ complex on spectrum of absorbance correspond for 580 nm \[3\]. 8PTSQ is very sensitive for revealing of Zn\(^{+2}\)-ions in solutions contained minimal concentrations as \(10^{-7}\)–\(10^{-8}\) of Zn\(^{+2}\)-ions and is used for color revealing of its in solutions. Diabetogenic properties of all these substances were established previously and determined by ability to form complex salt with Zn\(^{+2}\)-ions in cytoplasm of B-cells that result necrosis and death of cells within short time \[4, 5\]. Using of transmission electron microscopy it was confirmed \[6\] that death of B-cells determinad by destruction of B-granules of B-cells, contained maximal concentrations of Zn\(^{+2}\)-ions. Injection 35–50 mg per kg body weight of these substances result developing of type 1 diabetes in animals within 1–3 days and accompanied by destruction of B-cells, marked increasing of blood glucose level until 20–25 mM as by developing of evident histological changes in pancreatic islets typical for type 1 diabetes \[2, 3, 6\].

**Aim of work:** investigate interaction of Zn\(^{+2}\)-ions contained in B-cells with 8PTSQ and DZ in pancreas tissue of intact animals as in Zn\(^{+2}\)-ions in animals with type 1 diabetes, past elimination of ions from B-cells and past preliminary concurrent binding of ions with chemicals possess more high affinity for Zn\(^{+2}\)-ions.

**Methods.** 14 Rabbits 2450–2850 g, 26 Wistar Rats 165–176 g and 8 Mices 33–38 g were used.

1. Experiences with Dithizon and XA. Preparing of Dithizon solution: 30 mg of Dithizon, (SIGMA, USA) +10 ml bidistillate+0.2 ml 25 % NH\(_4\)OH 10 min mixing on temperature +70° at Celsium. Solution was injected intravenously to Rabbits and to Mices 46–48,6 mg/kg. Diabetes caused by XA was produced by containing of Rats on diet stimulated endogene synthesis of XA in animals and Human \[3, 7\].

2. Experiences with 8PTSQ. Preparing of 8PTSQ solution: 25 mg of 8PTSQ (Inst. High Pure Chemicals, Moscow) was dissolved in 65 % Ethanol on +70° Celsium and injected to Rabbits 35,5–38,8 mg/kg \[8\].
3. Experiences with Na salt of Diethylthiocarbamic Acid [DDCA], a blockator of Zn$^{2+}$-ions in B-cells [8]. DDCA formed not toxic for B-cells complex with Zn$^{2+}$-ions and not result developing of experimental diabetes [9]. Contrary, binding of Zn$^{2+}$-ions by DDCA, injected in doses as 500–1000 mg per kg body weight in 95 % animals protect B-cells of death and of developing of diabetes caused by DZ and diabetogenic derivatives of 8-oxyquinolin for 12–24 h [5]. We used water solution of DDCA (MERCK, Germany) which was injected to Rabbets for 42,3–46,2 mg/kg.

4. Experiences with removing of Zn$^{2+}$-ions from B-cells by Glibenclamide [GB] and with extraction of complex Zn$^{2+}$-DZ from B-cells. Mobilisation of Zn$^{2+}$-ions from B-cells protect cells of death caused by chelatotors [10, 11]. Suspension of GB in starch was used for per oral injections of GB 1,2 mg/kg daily within 3 days. 6 Rats were used. Frozen sections of Rat’s Pancreas 4 mcm were investigated 10 min past injection on dark microscopy. Intensity of staining was measured by photometer. 2nd part of pancreas tissue was fixed in Ethanol 70 %; paraffin sections of tissue were stained by 0,4 % acetone solution of 8PTSQ [6, 12, 13, 14] and investigated on fluorescent microscope. 3rd part of Pancreas tissue was fixed in Bouin 24 h. Staining technologies: staining of paraffin sections of pancreas 4 mcm by Aldehyde-fuchsin and immunohistochemical technics. Vital interaction of Zn$^{2+}$-ions-8PTSQ, negative reaction past injection of 50 mg/kg of DDCA [Fig. 1: 1.9] and partial decomposition of complex Zn$^{2+}$-ions-DDCA 6h past injection [Fig. 1: 1.8, 1.9]. Extraction of DZ-Zn$^{2+}$ complex from B-cells result negative reaction for Zn$^{2+}$-ions by both methods [Fig. 1: 1.7–1.9].

Animals with type 1 Diabetes. It is known that as Dithizone as multiple diabetogenic derivatives of 8-oxyquinolin caused developing of heavy diabetes 1 day past administration. Blood Glucose level first a few hours is decreased as result of releasing a large amount of insulin from destroyed B-cells and 24h later is increased till 15–25 mM (Fig. 5). Contrary, in animals with XA-diabetes blood Glucose level slowly increased till 9,0–12,0 mM (Fig. 6). Histostructure of pancreatic islets of animals with DZ-diabetes and 8PTSQ-diabetes: necrosis and destruction of B-cells as disappearing of Zn$^{2+}$-ions and deposited insulin from cytoplasm of cells [Fig. 1 1.13–1.15; Table 1]. In opposite, not so marked decrease amount of insulin as of Zn$^{2+}$-ions con tent in B-cells of pancreas tissue of animals with XA-diabetes were observed [Fig. 1: 1.4; 1.5; Ta ble 1].

Discussion

It is known that B-cells of Pancreas tissue of Human, Rabbits, Dogs, Mice, Rats, Kats contained a large amount of Zn$^{2+}$-ions which formed into cytoplasm of B-cells deposited form of insulin located in B-granules. Elimination of insulin from cells accompanied by decomposition of Zn$^{2+}$-Insulin complex [15]. Deposited insulin concentrated on apical part of B-cells contacted with islet’s blood vessels. Thus, B-cells formed around blood vessels a ring of cells, contained maximal concentrations of deposited insulin (Fig. 1: 1.4) well visible on sections stained by aldehyde-fuchsin and immunohistochemical technics. Vital interaction of Zn$^{2+}$-ions in B-cells with DZ result forming granules of Zn$^{2+}$-DZ located on apical part of cells too. Previously on parallel sections of pancreas tissue it was showed identical localization as of deposited insulin as of Zn$^{2+}$-DZ complex in cytoplasm of B-cells. It was evidently showed that diabetes caused by DZ is developed in animals in case if past injection of DZ complex Zn$^{2+}$-DZ formed in B-cells only and never developed if this red complex is not formed due to any causes. What part of amount of Zn$^{2+}$-ions in cytoplasm of B-cells reacted with DZ past injection of diabetogenic dose? Our previous works [2, 5] showed that past extraction from B-cells of Zn$^{2+}$-DZ complex by CHCl$_3$ we found a complete absence of Zn$^{2+}$-ions in B-cells (Fig. 1: 1.13–1.15) as of deposited insulin. Thus, these data showed that all amount of Zn$^{2+}$-ions contained in cytoplasm of B-cells reacted with DZ past one injection and complete disappearing of Zn$^{2+}$-ions from B-cells is accompanied by complete disappearing of insulin from cells too. This result demonstrate that histochemical reaction with DZ revealed not only Zn$^{2+}$-ions in cytoplasm of B-cells but deposited insulin too.
Fig. 1. Histotopography
In Fig. 1:
1. Frozen section of intact Rabbit’s Pancreas. Dark microscopy: A-cells on periphery of islet, B-cells in central part; ×280;
2. Frozen section of Rabbit’s Pancreas past injection of 48,9 mg/kg of DZ. Dark microscopy: red granules of Zn\textsuperscript{2+}-DZ complex in B-cells; ×280;
3. Frozen section of Mice Pancreas past injection of 50,8 mg/kg of DZ: granules of Zn\textsuperscript{2+}-DZ complex around blood vessels; ×280;
4. Section of intact Rat’s Pancreas. Staining by Aldehyde-fuchsin: violet color of B-cells determined by large amount of deposited insulin in B-cells. Histosstructure without changes; ×280;
5. Section of Pancreas of Rat’s with diabetes caused by XA. Aldehyde-fuchsin: necrosis and destruction of B-cells, decrease of insulin content; ×280;
6. Section of Pancreas of Rat’s with diabetes caused by XA. Fluorescent staining of Zn\textsuperscript{2+}-ions: decrease of concentration of Zn\textsuperscript{2+}-ions in B-cells; ×140;
7. Section of intact Pancreas of mice. Fluorescent staining of Zn\textsuperscript{2+}-ions. High concentration of Zn\textsuperscript{2+}-ions in B-cells; ×140;
8. Section of Pancreas of Rabbit’s past injection of DDNa. 250 mg/kg. Fluorescent staining of Zn\textsuperscript{2+}-ions: decrease of intensity of fluorescence as result of binding of Zn\textsuperscript{2+}-ions in B-cells by DDNa; ×140;
9. Section of Pancreas of Rat with XA-diabetes. Negative reaction for Zn\textsuperscript{2+}-ions in B-cells as result of destruction cells and disappearing of ions; ×140;
10. Frozen section of Rabbit’s Pancreas past injection of 51,3 mg/kg of DZ. Dark microscopy: granules of Zn\textsuperscript{2+}-DZ complex in B-cells; ×280;
11. Same Frozen section of Rabbit’s Pancreas past extraction of Zn\textsuperscript{2+}-DZ complex from B-cells by chloroform. Dark microscopy: granules of Zn\textsuperscript{2+}-DZ complex disappeared (extracted) from B-cells; ×280;
12. Same Frozen section of Rabbit’s Pancreas past injection: Zn\textsuperscript{2+}-reaction with 8PTSQ is negative, fluorescent microscopy; ×140;
13. Frozen section of Rabbit’s Pancreas past injection of 47,6 mg/kg of DZ. Dark microscopy: granules of Zn\textsuperscript{2+}-DZ complex in B-cells; ×280;
14. Frozen section of Rabbit’s Pancreas with diabetes caused by DZ. Dark microscopy: absence of Granules of Zn\textsuperscript{2+}-DZ complex in B-cells as result of destruction of B-cells; ×280;
15. Same islet. Negative fluorescent reaction for Zn\textsuperscript{2+}-ions as result of death of B-cells an disappearing of Zn\textsuperscript{2+}-ions; fluorescent microscopy; ×140.

Table 1

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<tr>
<th>№</th>
<th>Zn\textsuperscript{2+}-ions and insulin content in B-cells stained by Dithizon, 8PTSQ and Diethylpseudoisocyanine</th>
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<tr>
<td></td>
<td>Intact animals</td>
</tr>
<tr>
<td>1</td>
<td>Dithizon (DZ)</td>
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<tr>
<td>2</td>
<td>8PTSQ</td>
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<td>3</td>
<td>Diethylpseudoisocyanine</td>
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Fig. 2. Zn\textsuperscript{2+}-DZ complex content in B-cells

Fig. 3. Zn\textsuperscript{2+}-8PTSQ complex content in B-cells

1, 3, 5, 7 — intacts, not staining;
2— intact, staining by DZ; 4 — DZ-diabetes;
6 — 8PTSQ diabetes; 8 — XA-diabetes
Experiences with 8PTSQ. 8PTSQ is a high specific fluorescent reagent for revealing minimal concentrations of Zn\(^{2+}\) ions in solutions as \(10^{-7} - 10^{-8}\). This same time due to the ability to form in B-cells of toxic complex as Zn\(^{2+}\)-ions-8PTSQ this reagent is possess diabetogenic properties as other derivatives of 8-oxyquinolin and is able in doses as 30–35 mg per kg to provoke developing of heavy diabetes in 100 % of animals within 1–2 days past injection. For staining of frozen sections of pancreas tissue Zn\(^{2+}\)-ions in cytoplasm of B-cells we used a few drops of 0.4 % 8PTSQ acetone solution for 1 min. We observed Zn\(^{2+}\)-ions as intensive green fluorescence in cytoplasm of B-cells of intact animals and as absence of fluorescence in animals with diabetes Zn\(^{2+}\)-ions (Fig. 1: 1.6, 1.7, 1.9).

Despite of fact that usually there are parallelism of content Zn\(^{2+}\)-ions and insulin in B-cells, is not possible to use results of reaction with 8PTSQ for estimate amount of insulin in B-cells as by DZ-technic because 8PTSQ as chemical reagent revealed pure Zn\(^{2+}\)-ions in B-cells but not complex Zn\(^{2+}\)-ions-chelator. In added, 8PTSQ-technic is valid for revealing of Zn\(^{2+}\)-ions in B-cells as free ions or ions formed complex with insulin. In case if insulin is connected with DZ, or with derivatives of 8-oxyquinolin reaction with 8PTSQ be negative as in section of pancreas of diabetic animals despite normal amount of Zn\(^{2+}\)-ions n cells. Extraction of complex Zn\(^{2+}\)-DZ from B-cells (Fig. 1: 1.11) accompanied by disappearing of Zn\(^{2+}\) from B-cells. Same result we obtained in animals with diabetes: destruction of B-cells result disappearing of Zn\(^{2+}\) from cells and accompanied by negative reaction for Zn\(^{2+}\) in cells (Fig. 1: 1.13–1.15). Analogical negative result we showed in animals past injection of derivatives of Diethyldithiocarbamic acid (DDCA) which possess more high affinity for Zn\(^{2+}\) in compared with DZ and 8PTSQ [16]. As result DDCA remove DZ and 8PTSQ from com-
plexes Zn$^{2+}$-DZ and Zn$^{2+}$-8PTSQ with forming not visible and not toxic for B-cells complex Zn$^{2+}$-DDCA (Fig. 1: 1.9).

In conclusions, using noted above histochemical methods is possible to estimate as total amount of Zn$^{2+}$-ions as color complexes Zn$^{2+}$-insulin Zn$^{2+}$-DZ and Zn$^{2+}$-8PTSQ in cytoplasm of B-cells. In diabetic animals negative reaction for Zn$^{2+}$-ions demonstrate absence of ions as result of destruction and death of B-cells. Negative reaction in health animals showed absence of Zn$^{2+}$-ions in B-cells as result of elimination [16] caused by some drugs. This conditions in B-cells not able to make deposited form of insulin. Negative reaction in health animals caused by not long time binding of Zn$^{2+}$-ions by not diabetogenic chemicals demonstrate not absence of Zn$^{2+}$-ions in B-cells but presence in not visible complexes with chemicals. But in majority cases negative histochemical results revealing of Zn$^{2+}$-ions in B-cells are as sign of destruction and death of B-cells.

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References

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Диабет тудыратын кешентүшүн заттардың Zn\textsuperscript{+2} -иондарымен панкреаттык
В-клеткаларда ерекеттесуі және олардың В-клеткаларды бұзуға ықпал

Авторлар дитизондың және диабет тудыратын 8-оксихинолиндың тұңдылатының ерекеттесуін,
сондай-ақ адам ағашының түзілінін, панкреаттык В-клеткаларда қалыңған тұндылатын
және олардың жылдам бұзылатын 1-типті диабеттің дамуына әсерсіз сокырұғын зерттеген.
В-клеткаларда мәрім тұндылатын 8-оксихинолин ала алға шығуы ерекеттерін бұзымына немесе оларды
басқа диабет тудырмайтын кешентүшілер арқылы, оларды жоғарыда аталған диабет тудырмала
заттар компетен болдырмасы және жануарларда диабет тудырылуын қорғаған.

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Взаимодействие диабетогенных комплексообразующих веществ
с ионами Zn\textsuperscript{+2} в панкреатических В-клетках и их роль в разрушении В-клеток

Авторами изучено взаимодействие дитизона и диабетогенных производных 8-оксихинолина, включая
и образующиеся в организме человека, с ионами цинка, содержащимися в панкреатических В-клетках,
приводящих к быстрому их разрушению и развитию диабета 1 типа. Показано, что предотвращение
данного взаимодействия путем предварительного вымывания ионов цинка из В-клеток или блокиро-
вание их другими, недиабетогенными комплексообразователями предотвращает их взаимодействие
с названными выше диабетогенными веществами и развитие диабета у животных.

References