Research Article

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Histochemical Staining of Zn^{+2}-Ions and Insulin in B-Cells of Isolated Pancreatic Islets, Cells of Prostate and Sallivan Glands

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ABSTRACT

Keywords: Zn^{+2}-Ions Content in B-Cells; Pancreatic B-Cells; 8-Para (Toluenesulphonylamino) Quinolin (8PTSQ)

Background

Pancreatic B-cells contain a large amount of Zn^{+2}ions [1-3] as Salivary glands and prostate. In B-cells Zn^{+2}-ions take part in processes of biosynthesis of insulin as at processes of storage by forming of Zn^{+2}-insulin complex [4,5]. It is known that Zn^{+2}-ions in B-cells formed with insulin, a deposited form of hormone as Zn^{+2}-insulin complex [4]. Proinsulin forms a zinc contain hexamer soon after its synthesis. In addition, the Zn^{+2}-ions enhance proinsulin's solubility and render insulin insoluble. Zinc ions also appear to play an important role in the microcrystalline character of the precipitated insulin granule [5]. Pancreas of rat, rabbit, dog, cat, some fish, human, birds, mice, hamster, porcine, hoerst, contain a large amount of Zn^{+2}-ions [6]. Using of electron microscopy histochemical method, it was showed that that Zn^{+2}-ions are concentrated in B-cells in B-granules only contained deposited form of insulin [7] and destruction of B-cells caused by Dithizone which formed in B-cells toxic complexes with Zn^{+2}-ions, started by destruction of B-granules [8]. Very often in diabetes and intact B-cells there are a quantity correlation between insulin and Zn^{+2}-ions content: decreasing of insulin content accompanied by decreasing of amount of Zn^{+2}-ions and contrary in intact B-cells a large amount of insulin ac- companied by a large amount of Zn^{+2}-ions. For to estimate ability of B-cells for storage of insulin in cells it is necessary to use method of staining of Zn^{+2}-ions. Diabetogenic derivative of 8-aren (sulphonyl amino) quinoline as 8-para(toluenesulphonylamino)quiNolin [8PTSQ] and Diphenyl thiocarbazone (Dithizone)[DZ] possess high chemical affinity for Zn^{+2}-ions and in vitro formed color complexes as Zn^{+2}-8PTSQ [9,10] visible on fluorescent microscopy and Dithizone formed red Zn^{+2}-DZ complex visible as red granules using dark microscopy.

Aim of Work

1. To investigate Zn^{+2}-irons and insulin content in B-cells of isolated islets of animals as in pancreas tissue and in islets of pancreas of Human embryo 6-8 weeks old using staining by 8PTSQ, by Dithizone as by Aldehyde-fuchsin and Victoria 4 methods and immunohistochemical method.

2. To reveal Zn^{+2}-ions in tissues of Prostate and Salivary gland contained to a large amount of Zn^{+2}-ions.
3. To adopt all methods for modeling tissue culture of isolated pancreatic islets.

Methods. Materials

12 Rabbits 2450-2850 g., 10 Rats Wistar 162-175 g. and 5 mice 30-36 g.

Group 1: Diabetes induced by Dithizone was obtained by injection of water solution of Dithizone 48,8-50,4 mg/kg and of ethanol solution of 8PTSQ. Preparing Dithizone solution: 200 mg of Dithizone+15 ml of bidistillate+0,2 ml of 25% NH4OH+ 10 min. mixing on +70 0C water. Preparing of 8PTSQ solution: 25 mg. of 8PTSQ (Institute for High Pure Chemicals, Moscow) was dissolved in 65% Ethanol at +700 Celsius and was intravenously injected to Rabbits 38,5-40,8 mg/kg.

Group 2: Elimination of insulin and Zn^{+2}s-ions from B-cells by per oral administration of Gilgen- claimed, daily 20 mg/kg to Rats within 4 days.

Methods for Fixation of Pancreas

1. Pancreas tissue 24h in Blouin for light microscopy using staining by Aldehyde fuchsin and Victoria 4;
2. Isolated by collagenase islets 1h in Blouin.
3. Fixation of pancreas tissue and of isolated islets in 700 ethanol+H2S for fluorescent method staining of Zn+2-ions by using 8PTSQ.

Group 3: Isolated pancreatic islets of neonatal Rats: In my experiences 18 neonatal infant rats of LEWIS and 14 neonatal Wistar Rats were used. Isolation of pancreatic islands is carried out using of 2% solution of Collage- nase by Lacy R. and Kostianovsky M. (11). Separation of islets using in two ways:

1. Separation in gradient of density of Dextran.
2. Manual selection of islets. The islets were pre-cultivated for 3 hours medium of RPMI-1640 + 5 mmol/l of glucose and embryonal bovine serum.

Staining Technologies

1. Staining of Zn^{+2}-ions in sections of pancreas tissue of rabbits, rats and mice using staining by 8PTSQ and by Dithizone of Zn^{+2}-irons and of insulin by Aldehyde-fucshine, Victoria 4 as by Immunohistochemical and Pseudoisocyanine methods.
2. Revealing of Zn^{+2}-ions in sections of tissues of Prostate and Salivary glands of intact Rabbits
3. using staining by 8PTSQ of Zn^{+2}-ions. 0,04% acetone solution of 8PTSQ (IREA, a Instituite for High Pure Chemicals, Moscow, Russia) was used.
4. Revealing of Zn^{+2}-ions and insulin in B-cells of isolated pancreatic islets of Rats
5. Staining procedures using fluorescent reagent 8PTSQ: 0,04% acetone solution of 8PTSQ was used. Staining procedures: a few drops of 8PTSQ solution place on frozen sections for 10 sec.; 3 times washing by distilled water and investigation on UV-light microscope with measuring of intensity of fluorescence (control: intensity of fluorescence of exocrine cells was accepted for 1,00); length of wave of UV-light 360-370 nm.
6. Staining of insulin by Victoria 4, Immunohistochemical and Aldehyde fuchsin methods. For quantitative estimation of results of measuring intensity of fluorescence, the parameter K was calculated for:

Fluorescent Histochemical Methods

1. Fluorescent 8PTSQ method for staining of sections of isolated islets of animals: $K=IF_1/If_2$, whe- re: IF_1- intensity of fluorescence of B-cells in intact isolated islets; If_2, где: IF_2- intensity of fluores- cence of B-cells in isolated islets of diabetic animals.
2. Fluorescent 8PTSQ for islets of pancreas tissue of animals: $K=IF_1/If_2$, where: IF_1- intensity of fluorescence of B-cells; If_2, IF_2- intensity of fluorescence of exocrine tissue.

Aldehyde-Fucshine, Victoria 4, Dithizone Methods

1. For pancreas tissue: $K=AB_1/AB_2$ where: AB1-density (absorbance) of staining of intact B-cells; AB_2 - density of staining of exocrine tissue. 2.4. For isolated islets: $K=AB_1/AB_2$ where: AB_1-density of staining of intact B-cells; AB_2- density of staining of B-cells of diabetic isolated islets. Histofluorimetric complex was used for measuring intensity of staining (Figure 1). Figure 1. Histofluorimetric complex for quantitative measuring of intensity of fluorescence and of density of staining (Meyramov G.G., Tusupbekova G.T., Meyramova A.G.; Problemi endocrinologii, Moskva, 1987, vol..33, N6, P.49-51; Meyramov G.G., Kikimbaeva A.A., Meyramova A.G.Pre-Patent "Method for quantitative measuring of insulin content in pancreatic B-cells" N18352 dated by 18.01.2007., Ministry of Justice of Kazakhstan.

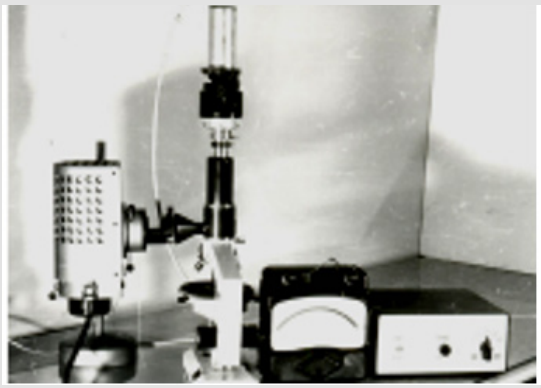

Figure 1: Staining of Zinc-ions and Insulin in B-cells of Pancreas tissue and in isolated pancreatic islets.

Results

Pancreas tissue of intact animals. Intensive fluorescence of complex Zn^{+2}-ions-8PTSQ in cytoplasm of B-cells of rabbits and mice was revealed as in sections of pancreas past staining by 8PTSQ solution as vital histochemical reaction (Figure 1.3) past intravenous injection of 8PTSQ. These results correlated with results of staining of insulin: a large amount of insulin was revealed in B-cells painted by Aldehyde-fucshine and Victoria methods (Figures 1.1 & 1.2, Table 1). Analogical results were obtained on a model of tissue culture of isolated islets. A large amount of insulin corresponds to the content of Zn^{+2}-ions in B-cells (Figures 1.4-1.6) of intact isolated islets. In the damaged islets the content of insulin considerably decreased up to its total disappearance (Figures 1.7 & 1.8). The content of Zn^{+2}-ions in B-cells of isolated islets decreased parallel to decrease in the content of insulin (Figure 1.9). There are a completely negative reaction for Zn^{+2}-ions in sections of pancreas of animals with experimental diabetes caused by selective destruction (Figure 1.9) of B-cells as past partial and complete elimination of Zn^{+2}-ions from B-cells by Glibenclamide (Figures 1.10-1.12; Table 1). Abscence of Zn^{+2}-ions in cytoplasm of B-cells was evidently confirmed in added by negative color reaction past staining of pancreas tissue by Dithizone (Figure 1.15, Table 1). The same results were obtained using vital staining of islets by Dithizone. We observed the very positive reaction for Zn^{+2}-ions in B-cells: a large amount of red granules of complex Zn^{+2}-Dithizone with maximum concentration in B-cells located around blood capillaries containing the greatest number of the deposited insulin as Zn^{+2}-insulin complex (Figures 1.13 & 1.14). Elimination of Zn^{+2}-ions from B-cells by Glipalamide accompanied negative reaction with Dithizone as complete absence of red granules of Zn^{+2}-Dithizone in cytoplasm of B-cells (Figure 1.15). Results of staining of frozen sections of tissues of Prostate and Sullivan gland evidently showed a presence of a large amount of Zn^{+2}-ions in cells confirmed by using of both methods (Figures 2.1-2.4) (Table 2).

Table 1: Insulin (IN) and zinc (Zn) content in B-cells of pancreas tissue (parameter K).

№	Experience Conditions	Content of insulin and zinc in B-cells of pancreas tissue (parameter K)				
		Insulin (IF) staining by Pseudoi- socyanine method	Insulin (AF) staining by Aldehy- defucshine meth- od	Insulin (V4) staining by Dime- thylnaphtylmethan (Victoria-4) method	Insulin (IM) staining by Immu nohistochemi- cal method	Zn-ions staining by 8PTSQ fluor-ochrom (Zn)
1	Intact animals	1,96±0,08●	1,84±0,06*	1,90±0,11+	1,82±0,03×	2,04±0,05●*
2	Dithizon indu- ced-diabet4es (48,8-51,6 mg/kg)	1,04±0,02 p<0,001 n-18	1,29±0,04 p<0,01 n-22	1,53±0,09 p<0,05 n-24	1,06±0,02 p<0,001 n-19	1,05±0,03 p<0,001 n-26
3	Elimination of Zn-ions from B-cells	1,09±0,04● ●-p<0,001 n-20	1,14±0,05* *-p<0,001 n-20	1,26±0,08+ +-p<0,01 n-28	1,04±0,02× +-p<0,01 n-16	1,02±0,04●* ●*-p<0,001 n-19

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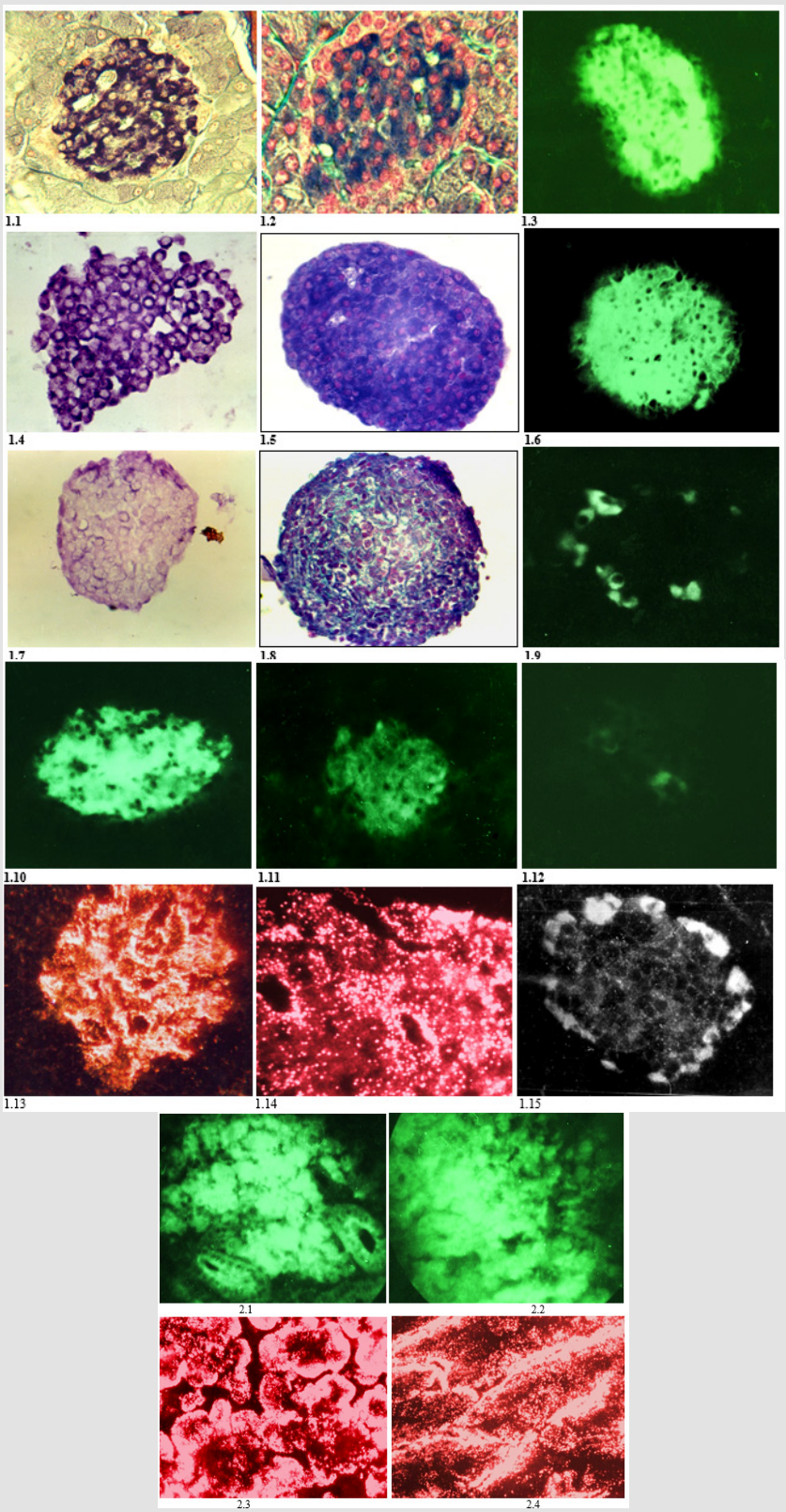

Figure 2: Staining of Zn+2-ions in tissues of Prostate and Sallivan glands.

Table 2: Insulin (IG) and Zinc content (IF) in B-cells of isolated islets (parameter K).

№	Conditions	Insulin (IG) and Zinc content (IF) in B-cells of Isolated islets (parameter K)				
		Insulin (IF) pseudoisocyanine	Insulin (AB) aldehyde-fucshine	Insulin (AB) Victoria 4	Immunohisto-chemical method (AB)	Zn^{+2}-ions (IF)
1	Intact animals	1,83±0,04+	1,83±0,04●	1,91±0,04×	2,02±0,05*	2,02±0,05*
2	Diabetic animals	1,04±0,01+	1,18±0,02●	1,35±0,06×	1,02±0,01*	1,02±0,01*

Figure 1 Staining of Zinc-Ions and Insulin In B-Cells of Pancreas Tissue and In Isolated Pancreatic Islets. Pancreas of intact Rat. Aldehyde-fucshine; x280; 1.2. Pancreas of intact Rat. Victoria 4; x280; 1.3. Pancreas of intact Rat Staining of Zn^{+2}-ions by 8PTSQ; x140; 1.4. Isolated islet of Rat Aldehyde-fucshine; x280; 1.5.Isolated islet of Rat Victoria 4; x280; 1.6.Isolated islet of Rat Staining of Zn^{+2}-ions by 8PTSQ;x140; 1.6. Frozen section of Pancreas of diabetic Rat Staining of Zn^{+2}-ions by 8PTSQ: absence of fluorescence as result of destruction of B-cells and absence of Zn^{+2}-ions; x140; 1.7. Isolated islet, Aldehyde-fuchsin. Destruction of B-cells, negative reaction for insulin; x280; 1.8. Isolated islet, Victoria 4. Destruction of B-cells, negative reaction for insulin; x280; 1.9. Isola ted islet; staining by 8PTSQ; negative reaction for Zn^{+2}-ions; x140; 1.10. Isolated intact islet.; staining by 8PTSQ; positive reaction for Zn^{+2}-ions in B-cells; x140; 1.11. Isolated islet. Partial elimination of Zn^{+2}-ions from B-cells by Glibenclamide; staining by 8PTSQ; weakening of fluorescence of B-cells;x140; 1.12. Isolated islet. Complete elimination of Zn^{+2}- ions from B-cells; staining by 8PTSQ; negative reaction for Zn^{+2}-ions; x140; 1.13. Frozen section of Pancreas of Rabbit past injection of Dithizone (DZ) 48,9 mg/kg.

Red granules of complex Zn^{+2}-DZ in B-cells. Darc microscopy; x280; 1.14. Frozen section of Pancreas of Mice past injection of Dithizone 49,5 mg/kg. Red gran-ules of complex Zn^{+2}-DZ in B-cells;Darc microscopy; x280; 1.15. Frozen section of Pancreas of Rabbit past complete elimination of Zn^{+2}-ions from B-cells by Glipalamide and followed injection of Dithizone,50,6 mg/kg.; absence of complex Zn^{+2}-8PTSQ in B-cells; Darc microscopy; x280 2.1. Frozen section of Prostate tissue of Rabbit. Staining of Zn^{+2}-ions by 8PTSQ; Positive reaction for Zn^{+2}-ions; x140; 2.2. Frozen section of tissue of Salivary gland of Rabbit; Staining of Zn^{+2}-ions by 8PTSQ; Positive reaction for Zn^{+2}-ions; x140; 2.3. Frozen section of Prostate tissue of Rabbit. Staining of Zn^{+2}-ions by Dithizone; red granules of complex Zn^{+2}-DZ in cells; x280; 2.4. Frozen section of tissue of Salivary gland of Rabbit; Stainining of Zn^{+2}-ions by Dithizone; red granules of complex Zn^{+2}-DZ in cells; x280.

Discussion

In 1961 Boshevolnov E. and Serebryakova G. informed about ability of 8PTSQ, a derivative of 8-oxyquinolin, to form in vitro complexes with Zn^{+2}-ions and with ions of Cadmium (Figures 3a & 3b). One of these chemicals, a 8-para(toluene sulphonyl amino) quinoline [8PTSQ] for- Ming complex 1:1 with Zn^{+2}-ions as Zn^{+2}-8PTSQ induces green fluorescence under UV-light 360-370 nm length. 8PTSQ is a high specific fluorescent reagent for identification of very small amounts of Zn^{+2}-ions in solutions and tissues in concentrations as 10-7-10-8. It was confirmed by using of spectral analysis that maxi mum of absorbance of spectrum of absorbance of extracted from B-cells complex Zn+2-8PTSQ equal 530 nanometers completely correspond to maximum of absorbance of the pure complex Zn^{+2}-8PTSQ synthesized in vitro. Later the histochemical method for fluorescent identification of Zn^{+2}-ions in pancreatic B-cells was elaborated and adapted for frozen sections of pancreas tissue. Meanwhile this method was not adopted for using sections of fixed pancreas tissue as for model of isolated pancreatic islets. Our results have allowed us to adapt this method for using of the fixed pancreas tissue and of tissue culture model of isolated pancreatic islets. For to adapt this histochemical method for using of sections of fixed tissue of pancreas, fixation was carried out not in Blouin liquid which interferes with reaction, but in 700 ethanol enriched by H2S.

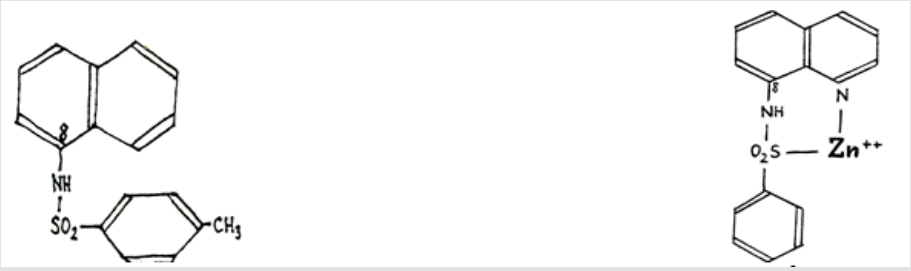

Figure 3:
a. 8-para(toluenesulphonylamino) quinolin (8PTSQ)
b. Complex Zn+2-8-para(toluenesulpho-nylamino)quinolin.

There are some difficulties on process of filling suspension of isolated islets in paraffin, de- termined by need evenly to disseminate islets them on all volume of the paraffin block, without having given the chance to settle on a bottom or to take place in one layer. For this purpose, paraffin was filled in glass test tube where the needle of the syringe containing the fixed islets in the minimum volume of a nutrient medium was entered above 3-4 mm of a bottom of the test tube. Then suspension was mobilized from syringe under visual control at his simultaneous slow rise vertically syringe to level below 4-5 mm from the upper bound of paraffin in a test tube. The advantage of the used by us a Dithizone method for staining Zn^{+2}-ions is its ability to form bright red granules of a complex Zn^{+2}-Dithizone of structure 2:1 that giving a good possibility to investigate a metal gist topography in pancreatic islets. Due to this feature, it was showed that maximum amount of granules of Zn^{+2}-Dithizone complex concentrates in B-cells located around blood capillaries in islets on the pole of cells contacted with capillar's wall.

This method also is high specific for staining of Zn^{+2}-ions in B-cells:

1. Maximum of absorbance of the complex Zn^{+2}-Dithizo- ne extracted from B-cells correspond to maximum of absorbance of pure complex synthesized in vitro;

2. Ions of other metals with which Dithizone potentially could form a complex in B-cells are absent. It is known that besides B-cells of pancreas, tissues of prostate and salivary glands contain a large amount of Zn^{+2}-ions. Our results allowed us to reveal in cells of both glands a Zn^{+2}-ions using both histochemical methods. All microphotographs prepared by Prof. G.G. Meyramov from histol. slides prepared by him in experiences due to financial supporting of family (1977-2018).

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