UDC 613.2:612.359:616–012

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High specific fluorescent method staining of zinc-insulin complex in pancreatic B-cells

Authors demonstrated results using of high specific fluorescent method histochemical staining of Zn^{+2}-ions in pancreatic B-cells. It was showed in diabetic animals and in animals past mobilization of insulin from B-cells a simultaneous decreasing as of amount of insulin as of Zn^{+2}-ions in cytoplasm of B-cells. Meanwhile formation in B-cells of chemical complexes of derivatives of Diethyldithiocarbamic acid with Zn^{+2}-ions result negative fluorescent reaction for Zn^{+2}-ions but positive reaction for insulin using of insulin staining methods.

Key words: insulin contents, immunohistochemical, immunofluorescent, pseudoisocyanine and aldehyde fuchsine methods, pancreatic B-cells.

It is known that pancreatic B-cells contained a large amount of ions of Zinc [1–3] as salivary glands and prostate. In B-cells zinc ions take part in processes of biosynthesis of insulin as in of storage of insulin by forming of zinc-insulin complex [4, 5]. Pancreas of rat, rabbit, dog, cat, some fish, human, birds, mice, hamster, porcine, hoerst, contained a large amount of zinc. Using of electron microscopy histochemical method it was showed that that zinc concentrated in B-cells in B-granules only contained deposited form of insulin [6] and destruction of B-cells caused by Dithizon which formed in B-cells toxic complexes with zinc-ions, started by destruction of B-granules [7].

Widely known methods staining of insulin as immunohistochemical, immunofluorescent, diethylpseudoisocyanine and some other methods are specific for insulin only but not for staining of zinc ions. Very often in diabetes and intact B-cells there are is a quantity correlation between insulin and zinc ions content: decreasing of insulin content accompanied by decreasing of amount of zinc ions and in opposite, in intact B-cells a large amount of insulin accompanied by a large amount of zinc-ions. Meanwhile for estimate ability of B-cells for storage of insulin in cells it is necessary to use method of staining of zinc-ions whereas staining of insulin is indirect method for to estimate concentration of zinc ins in B-cells.

Aim of work: to study result using of high specific fluorescent methods revealing of zinc ions by using of 8-para(toluenesulphonylamino)quinolin (TSQ), a high specific for Zn^{+2}-ions reagent [8, 9] which formed complex «zinc-TSQ». TSQ is a derivative of 8-oxyquinolin and synthesis was elaborated by Prof. N.N.Voroshzov in 1930 [10]. In UV-light with maximum of absorbance as 360–370 nm, this complex fluoresces brightly green light [11]. Specificity for zinc ions of this method was confirmed in vitro by interaction of pure zinc ions with TSQ that result intensive green fluorescence of solution; using of spectral analysis it was confirmed presence in solution of «zinc-TSQ» complex and correlation of maximum of absorbance of this complex with pure complex synthezed in vitro. Sensitivity of this method is high and concentration of zinc as 10^{-7}–10^{-8} revealed using it [8]. This same time in TSQ is TSQ possess a high diabetogenic activity and injection of 40–50 mg/kg result necrosis, destruction and death of absolute majority of B-cells within 20–30 min past formation of complex «zinc-TSQ» in B-cells [12].

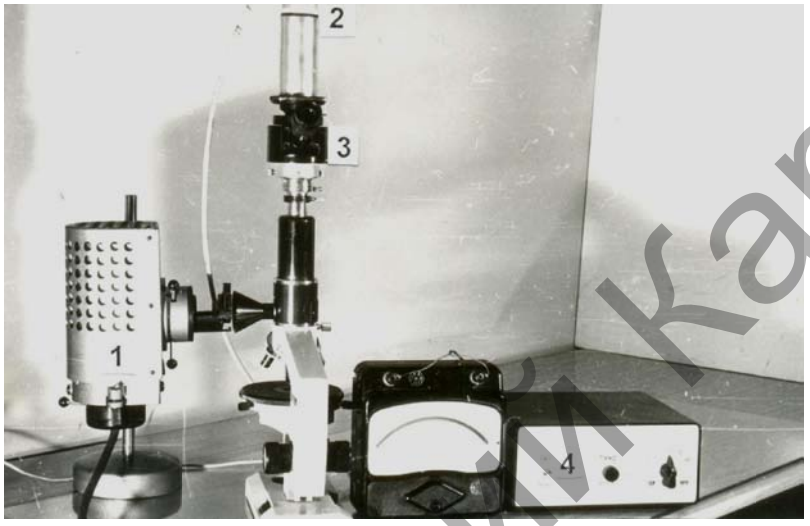
Materials and methods

8 rabbits (2450–2680 g) and 3 Guinea Pigs (320–370 g). Group 1: 3 animals with diabetes caused by Dithizon (48,8–51,6 mg/kg); Group 2: intact rabbits; Group 3: 3 animals past administration of Na salt of diethyldithiocarbamic acid (DCA) (504 and 987 mg/kg) that result temporary non toxic binding of zinc in B-cells; Group 4: 2 Guinea Pigs past administration of DZ (49,5–52,8 mg/kg) and 2 intact Guinea Pig.

Preparing of Dithizon water-ammonia solution: Dithizon (Avocado Chem. Company, USA) — 200 mg.+ 15 ml. of bi-distilled water + 0,25 ml of 25 % ammonia, 10 min mixing on temperature +70º Celsius.

Staining technologies

1. Fluorescent reaction with TSQ. 0,04 % ammonia solution of TSQ was used. Staining procedures: a few drops of TSQ solution place on frozen sections for 10 sec.; 3 times wash by distilled water and investigation on UV-light microscope with measuring of intensity of fluorescence (intensity of fluorescence in control was accepted for 1,00; length of wave of light — 360 nanometers. For quantitative estimation of results of measuring intensity of fluorescence parameter K was calculated as relation: Intensity of fluorescence of B-cells IF1/ Intensity of fluorescence of exocrine tissue cells IF2 (IF1/IF2); intensity of staining of exocrine tissue cells was accepted for 1,00 using of histofluorimetrical complex constructed by G.G.Meyramov and coll. [13] (Fig. 1).



1 — UV-lamp; *2* — photo-electronic multiplier; *3* — diaphragm for a choice of B-cells; *4* — electric device

Figure 1. Histofluorimetrical complex for measurement amount of zinc-insulin complex in pancreatic B-cells

2. For insulin staining the Immunohistochemical (anticorps for insulin from DAKO, Denmark) and Pseudoisocyanine [14] (SERVA, Germany) methods was used. For quantitative estimation of results of measuring intensity of fluorescence parameter K was calculated as relation: Intensity of fluorescence of B-cells IL1/ Intensity of fluorescence of exocrine tissue cells IL2 (IL1/IL2); intensity of staining of exocrine tissue cells was accepted for 1,00. For quantitative estimation of results of measuring density of staining B-cells by Immunohistochemical method parameter K was calculated as relation: Density of staining of B-cells IG1/ Density of staining of exocrine tissue — IG2 (IG1/IG2); intensity of staining of exocrine tissue cells was accepted for 1,00.

For histological analysis Victoria-4 histochemical method (MERCK, Germany) was used [15–18].

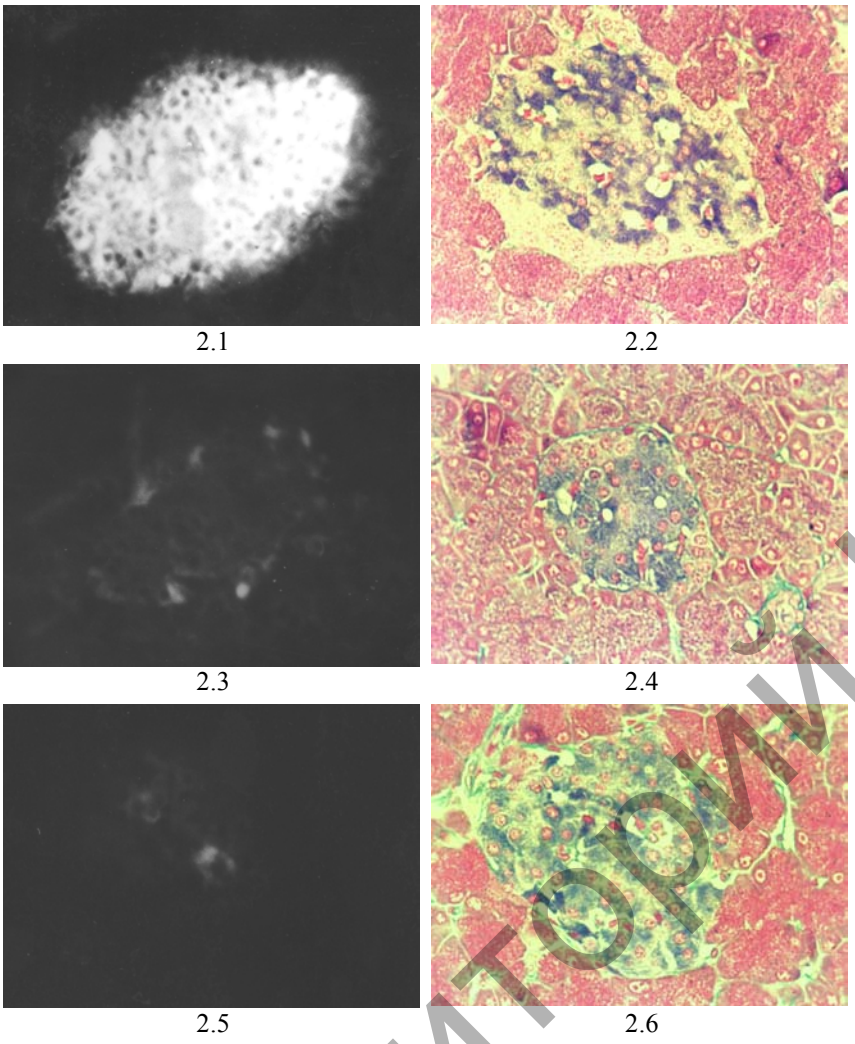
Results

1. Group 1. Immediately past injection of Dithizon (DZ) negative reaction for zinc ions was revealed in B-cells contrary to positive reaction for insulin (fig.2.1, 2.2) that is determined by binding of all amount of zinc in cells by DZ. As result zinc ions not formed with TSQ visible fluorescent complex in cytoplasm of B-cells. Intensity of fluorescence of B-cells: K(IF1/IF2) = 1,04±0,02; control: intact B-cells: K = 2,02±0,07 ($p < 0,001$). Insulin content in B-cells: K(IG1/IG2) = 1,88±0,02 IL1/IL2 = 2,06±0,08 (Table).

2. Group 2. Animals with diabetes caused by injection of DZ (50,2 and 47,6 mg/kg) 7 days ago. Negative reaction as for zinc-ions with TSQ as for insulin revealed in B-cells on frozen sections of pancreas tissue (fig. 2.3, 2.4) that demonstrated absence in cytoplasm of B-cells as of zinc-ions as of insulin in result of necrosis and destruction of cells: K(IF1/IF2) = 1,08±0,03; control: intact B-cells: K = 2,00±0,08 ($p < 0,001$). Insulin content in B-cells: K(IG1/IG2) = 1,12±0,02; IL1/IL2 = 1,07±0,06 (Table).

3. Group 3. Injection of Na salt of diethyldithiocarbamic acid (DCA) result forming in cytoplasm of B-cells of not toxic complex zinc-DCA for a few hours. A negative reaction for zinc-ions (fig. 2.5) was revealed in B-cells past injection of DCA that is explained by formation of complex zinc-DCA that is why TSQ not formed fluorescent complex zinc-TSQ in cells [7]: K = K(IF1/IF2) = 1,02±0,04; control: intact

B-cells: K = 1,98±0,05 (p < 0,001). Positive reaction for insulin (fig. 2.6). Insulin content in B-cells: K(IG1/IG2) = 1,85±0,04; IL1/ IL2 = 2,02±0,07 (Table).



2.1 — Pancreatic islet of intact rabbit. TSQ fluorescent reaction for zinc. Intensive fluorescence (a large amount of zinc in B-cells); UV-light microscopy; ×140;

2.2 — Pancreatic islet of intact rat. Victoria-4 staining method. Normal content of deposited insulin in B-cells (blue-violet color); ×280;

2.3 — Pancreatic islet of diabetic rats. TSQ fluorescent reaction for zinc. Completely negative reaction for zinc (absence of fluorescence) determined by destruction of B-cells and by absence of zinc-ions in cytoplasm; UV-light microscopy; ×140;

2.4 — Pancreatic islet of diabetic rats. Victoria-4 staining method. Decreasing of insulin content in B-cells and of size and number of islets in sections; ×280;

2.5 — Pancreatic islet of rabbit. DCA 987 mg/kg. TSQ fluorescent reaction for zinc. Completely negative reaction for zinc (absence of fluorescence) determined by not diabetogenic temporary binding of zinc-ions by DCA; UV-light microscopy; ×140;

2.6 — Victoria-4 staining method. Normal content of deposited insulin in B-cells (blue-violet color); ×280

Figure 2. Zinc-ions and insulin content in B-cells of intact and experimental rats

4. Group 4. B-cells of Guinea Pig contrary to many other animals (rabbit, rat, dog, fish, cat, hamster, porcine) not contained zinc ions and biochemical nature of processes of insulin storage in B-cells of Guinea Pig now not cleared yet.

Negative reaction for zinc-ions with TSQ was revealed in B-cells: K = (IF1/IF2) = 0,98±0,04; control: intact B-cells of rat: K = 1,97±0,06 (p < 0,001). Insulin content in B-cells: K(IG1/IG2) = 1,82±0,04; IL1/IL2 = 1,91±0,05 (Table).

T a b l e

Insulin and Zinc content in pancreatic B-cells (parameter K)

No.	Conditions of experience	Insulin (IG, IL) and Zinc content (IF) in B-cells (parameter K)		
		insulin (IG)	insulin (IL)	zinc (IF)
1	5 min. past injection of DZ	1,88±0,02*	2,06±0,08*	1,04±0,02
2	Diabetes caused by DZ (48,8–51,6 mg/kg)	1,12±0,02*	1,07±0,06*	1,08±0,03*
3	DCA (987 mg/kg)	1,85±0,04	2,02±0,07	1,02±0,04
4	Guinea Pig (intact)	1,82±0,04	1,91±0,05	0,98±0,04
5	Rabbit (intact)	1,92±0,04	2,02±0,06	1,98±0,06*

Note. * — p < 0,005.

Discussion

In 1961 E.Boshevolnov and G.Serebrarakova informed about ability of TSQ, a derivative of 8-oxyquinolin, to form in vitro complexes with Zn^{+2}-ions and with ions of Cadmium (Fig. 3, 4).

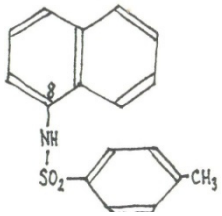


Figure 3. 8-para(toluene-sulphonylamino)quinolin (TSQ)

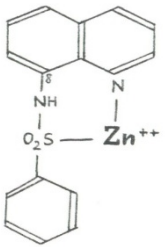


Figure 4. Complex Zn^{+2} – 8-para(toluene-sulphonylamino)quinolin (TSQ)

Zn^{+2}-TSQ complex radiates intensive green fluorescence under UV-light 360–370 nm length of wave and Cd-TSQ — intensive yellow fluorescence that was confirmed by spectral analysis of spectrum of absorbance. Past long time prolonging testing in Institute of High Pure Chemicals (Moscow) TSQ was proposed as fluorescent reagent for identification of very small amounts of zinc in solutions and tissues. Later in laboratory of Lasaris Y.A. and coll., Karaganda, TSQ was tested for revealing in vitro and in intact and diabetic animals of a large amount of zinc-ions. TSQ is high specific reagent for staining of zinc-ions in pancreatic B-cells. Now there are not other methods for revealing of zinc-ions in B-cells. It is known that zinc-ions take part in processes of storage of insulin by formation of complex zinc-insulin in B-cells. Very often there are parallelism between content of zinc and insulin in cytoplasm of B-cells and is possible to stain insulin in B-cells for estimate a content of zinc-ions in cells.

Results of using for many years of this method revealing of zinc-ions showed that in 3 cases method demonstrated a full coincidence with content of insulin in B-cells: 1) in intact animals; 2) in animals with experimental diabetes; 3) in animals after removing of zinc-insulin complex from B-cells by drugs. That is why this method can be used not only for estimate of zinc-ions content in B-cells but for insulin content too.

In one case results of TSQ-reaction can not correspond to quantity contained of zinc-ions in B-cells: some chemicals formed complexes with zinc in B-cells for short period and this time in fluorescent reaction for zinc will be negative despite of presence a large amount of metal in cytoplasm of cells.

This method demands following conditions: for fixing of tissue of a pancreas to use the alcohol sated with hydrogen sulfide (H_2S) or to use sections of frozen pancreas tissue. Filters for UV-microscopy: UV-filter between UV-lamp and microscope and yellow filter for ocular of microscope.

Acknowlegement

Authors are thankful for methodical assistance and financial supporting of investigation to Prof. G.G.Meyramov.

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Панкреаттық В-жасушаларда Zn^{2+} иондарын бояудың жоғары арнаулы гистохимиялық әдістері

Авторлар В-жасушаларда мырыш иондарын анықтайтын жоғары арнаулы люминесцентті әдісті қолданудың нәтижелерін көрсеткен. Бір мезгілде тәжірибелік диабетте В-жасушаларда және осы кешенді В-жасушалардан жұмылдыру кезінде де мырыш ионы мен инсулин мөлшерінің төмендейтіні көрсетілген. Сонымен қатар жасушаларда мырыш иондарының диэтилтиокарбоамин қышқылының туындыларымен байланысы — мырыш иондарына күрт кері әсер, ал инсулинге айқындалған оң әсер етуімен қатар жүреді.

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Высокоспецифичный гистохимический метод окраски ионов Zn^{+2} в панкреатических В-клетках

Авторами показаны результаты использования высокоспецифичного люминесцентного метода выявления ионов цинка в В-клетках. Отмечено, что одновременное параллельное снижение содержания ионов цинка и инсулина наблюдается в В-клетках при экспериментальном диабете и после мобилизации комплекса из В-клеток. Между тем связывание ионов цинка в клетках производными диэтилдитиокарбаминовой кислоты сопровождается резко отрицательной реакцией на цинк с сохранением хорошо выраженной положительной реакцией на инсулин.

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