UDC 616–018:616:379–008.64:577

G.G. Meyramov[1], A.S. Shaybek[1], G.T. Kartbaeva[1], E.M. Laryushina[2], G.O. Zhuzbaeva[1], A.R. Alina[2], O.-N.Dupont[1, 3], S.S. Tyrzhanova[1]

[1]Diabetes Research Group, Ye.A. Buketov Karaganda State University, Kazakhstan;
[2]Karaganda State Medical University, Kazakhstan
[3]Ocala, FL, USA;
(E-mail: meyramow@mail.ru)

Prevention destruction of pancreatic B-cells induced by chelators by reduced form of Glutathione

Authors investigated preventive effect of Reduced form of Glutathione on developing of diabetes caused by diabetogenic zinc binding ligands. It is shown that only Reduced form of Glutathione contains SH-radical in chemical structure possesses ability to protect B-cells of death unlike the Oxidized form of the Glutathione, free of it. It was established that injection of R-Glutathione, 1000 mg/kg completely connects with zinc in B-cells that protect cells of formation of toxic complexes causing destruction of B-cells and development of diabetes. Investigation of chemical aspect of interaction of R-Glutathione with zinc showed that administration of it result a complete blocking of zinc in B-cells therefore zinc does not revealed using of high specific and sensitive histochemical methods. The studying of duration of blocking of zinc in B-cells showed that complex Zn-R-Glutathione begins to dissociate with partial release of zinc approximately about 36 hours after injection and 48 hours later majority of complex is dissociate. Authors suppose that preventive ability of R-Glutathione is caused by ability to formation of not toxic temporary complex with zinc; zinc atom is fixed between atom of sulfur of the SH-radical and atom of oxygen or nitrogen which are contained in a molecule of R-Glutathione as well as after administration of diabetogenic chelators between of sulfur atom — and either oxygen atom, or atoms of nitrogen or carbon on the other hand.

Keywords: B-cells, R-Glutathione, insulin, zinc, experimental diabetes.

Abbreviations

R-Glutathione — Reduced form of Glutathione,
O-Glutathione — Oxidized form of Glutathione,
Zn — zinc,
DZ — Dithizon 8PTSQ — 8-para(toluenesulphonylamino)quinolin

Background

It was found that Diphenylthiocarbazone (Dithizon) and some derivatives of 8-oxyquinolin (8-ox) induced formation of toxic chelat complexes as «Zn-DZ» and «Zn-8-ox» in cytoplasm of B-cells that result selective destruction of B-cells within 15–30 min. and accompanied by developing of 1st type diabetes in animals [1]. Later it was reported preventive injection of some amino acids as Cystein and L-Hystidine contains sulfhydril SH-radical in structure of molecule, accompanied by protect B-cells of destruction caused by DZ and 8-ox that result prevention of developing of diabetes in majority of animals [2–5]. High durability of the Zn^{+2}-Dithizon complex 2:1 (Fig. 1, 2) determined by space elongation of molecule of Dithizon and disposition of two phenolic rings on the ends of a molecule that does not prevent the atoms of sulfur and nitrogen located in the center of a molecule to approach zinc atom. Besides, atom of zinc is located between two

atoms of nitrogen and sulfur, regarding to which affinity of zinc is very high and exceeds affinity to oxygen. [6]. It was supposed that protective activity of Cystein and L-Hystidine may be determined by the presence of SH-radical in the structure of molecule because formation of chelat complexes with DZ and 8-ox is processed by connection of Zn atoms with atom of S, H, O or N [6]. Aim of investigation: to study possible preventive effect of amino acids as Gluthation Reduced form contains SH-group and Gluthation Oxidised form not contains SH group.

Methods

Animals. 16 Rabbits 2400–2850 g.

Group 1. Injection of Dithizon, 48.6–51.2 mg/kg;

Group 2. Injection of Gluthatione-O, 970–1010 mg/kg + 10 min. later Dithizon, 49.8–50.6 mg/kg.

4 animals from each groups (1a and 2a) were killed 10 min. after injection of Dithizon and 4 animals (1b and 2b) — 9 days after injection.

Group 3. Injection of Gluthatione-R and Gluthatione-O: 985–1020 mg/kg. Animals were killed 6h, 12h and 24h after injection. Staining of frozen sections of pancreas for Zn-ions by Dithizon method and by 8PTSQ.

Frozen sections of pancreas of animals 1a and 1b groups were investigated using dark microscopy. Blood glucose level measuring — in animals of 1b, 2a and 2b groups before injection of Dithizon and 1, 3, 6 and 9 days after injection. Aldehyde-fucshine method [7–9] was used for analysis state of histostructure of pancreas tissue and Dithizon method formed red granules of complex «Zn–DZ» visible using dark microscopy. Maximum of absorbance of Zn^{+2}–DZ complex on spectrum of absorbance correspond for 530 nm [3].

8-para(toluenesulphonylamino)quinolin (8PSQ), a high specific fluorescent reagent was used for staining of Zn-ions in B-cells. 8TSQ formed fluorescent green complexes with Zn^{+2}-ions visible using fluorescent microscopy [10–12].

Results

Group 1a. Administration of DZ accompanied by formation of a large amount of red granules of Zn–Dithizon complex in cytoplasm of B-cells (Fig. 1). Maximal concentration of granules located on the pole of B-cells contacted blood capillaries that correspond to concentration of deposited insulin.

Group 1b. Experimental diabetes. Blood glucose concentration increased since 5.2±0.3 mM until 12.6 mM at 6^{th} day and 16.4±1.7 mM at 9^{th} day (Table 1). Histology: necrosis and destruction of 70–90 % of B-cells, marked decreasing of insulin and zinc content in B-cells.

Group 2a. Preliminary injection of Gluthatione-R result almost complete prevention of formation of «Zn-DZ» complex in B-cells (Fig. 1). Simple granules contacted capillaries contain granules of complex only.

Group 2b. Administration of Glutathione Reduced form before Dithizon accompanied by prevention developing of diabetes in 3 animals from 4 (Table 2, Fig. 1). In one rabbit (N3) blood Glucose level increase till 9^{th} day until 7.3. Histologic analysis showed decreasing of insulin content in cells without marked histological changes**.**

Administration of Glutathione Oxidized form before Dithizon not accompanied by prevention developing of diabetes: diabetes was developed in all animals (Table 3) with marked histological changes. Histologic analysis showed decreasing of insulin content in cells and presence of marked histological changes.

Group 3. It was important to obtain direct experimental confirmation that administration of Glutathione Reduced form to animals accompanied by binding of Zn-ions in B-cells as to investigate: how long time this complex exist in B-cells as we suppose, that 24 hours later after injection of R-Glutathione partial dissociation of complex is observed and 48 hours later there are almost complete dissociation (Fig. 1) with restoring of positive fluorescent reaction for zinc in B-cells.

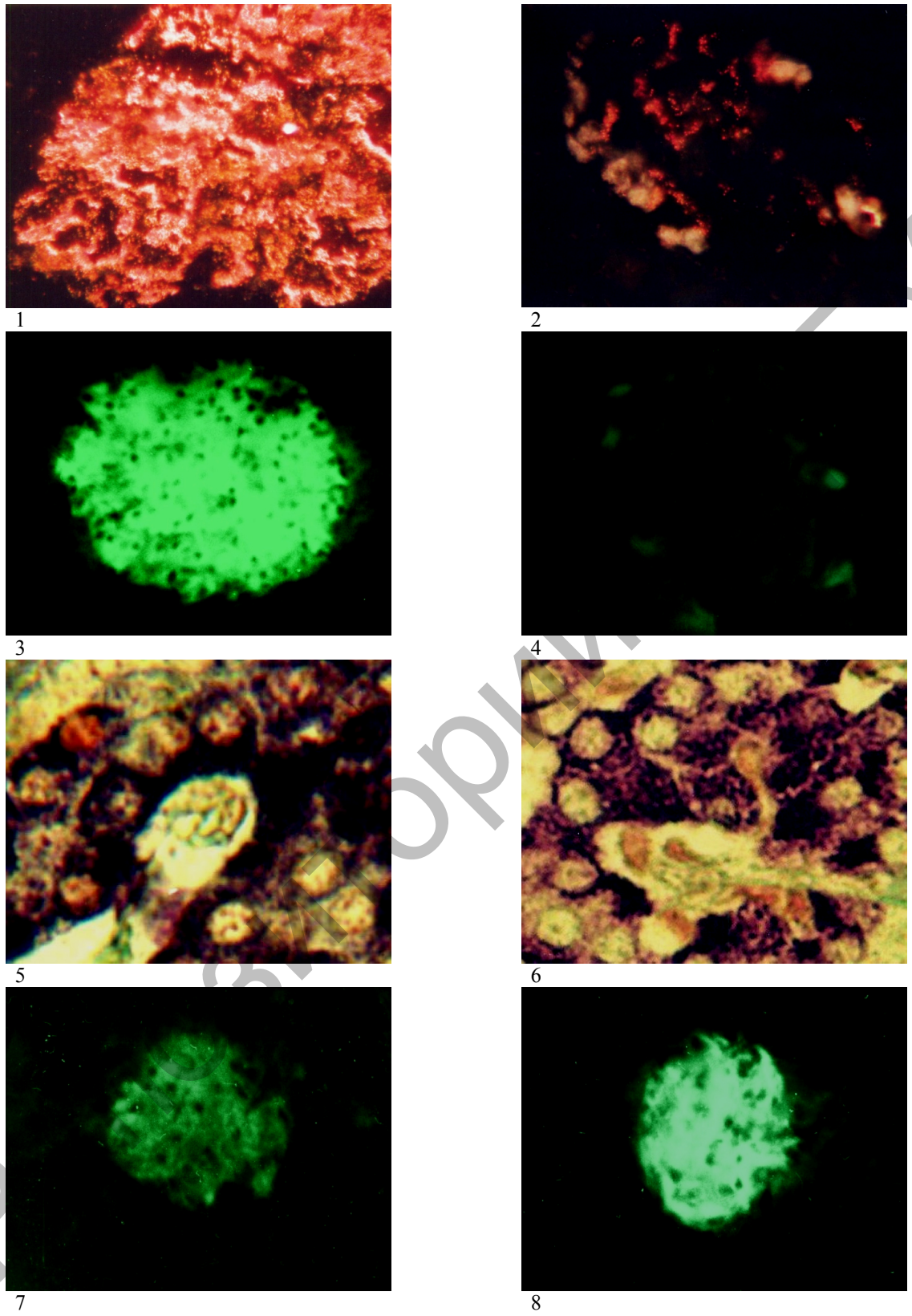


Figure 1

1 Pancreatic islet of intact rabbit. Dithizon, 48.9 mg/kg. Large amount of red granules of complex Zn-DZ in B-cells; Dark microscopy; ×280.

2 Pancreatic islet. Glutathione Reduced form, 985 mg/kg + Dithizon, 50,2 mg/kg. Almost complete absence of complex Zn-DZ in B-cells; Dark microscopy; ×280.

3 Pancreatic islet of intact rabbit. Positive fluorescent reaction for Zn-ions in B-cells; ×140.
4 Pancreatic islet. Glutathione Reduced form, 1015 mg/kg. Negative fluorescent reaction for Zn-ions in B-cells; ×140.
5 Pancreatic islet of intact rabbit. Histostructure and insulin content in B-cells without changes; Aldehyde-fucshine; ×280.
6 Pancreatic islet of rabbit. Glutathione Reduced form, 1010 mg/kg + Dithizon, 49.4 mg/kg .Histostructure and insulin content in B-cells without changes; Aldehyde-fucshine; ×280.
7 Pancreatic islet of rabbit. Glutathione Reduced form, 975 mg/kg, 36h after injection. Partial dissociation of complex Zn-Glutathione; Fluorescent reaction for Zn-ions; ×140.
8 Pancreatic islet of rabbit. Glutathione Reduced form, 1000 mg/kg, 60h after injection. Almost complete dissociation of complex Zn-Glutathione; Positive fluorescent reaction for Zn-ions; ×140.

Table 1

Blood glucose concentration in rabbits after injection of Dithizon

Animals	Dose of Dithizon (mg/kg)	Blood glucose concentration (mM)				
		before	1st day	3rd day	6th day	9th day
1	48,6	4,7	11,6	18,5	16,4	22,8
2	49,9	5,6	7,7	16,2	24,4	21,5
3	51,8	5,3	12,2	20,6	17,2	18,8
4	48,8	5,9	10,3	14,6	20,5	17,8

Table 2

Blood glucose concentration after injection of Glutathione Reduced form and Dithizon

Animals	Dose of Gluthation Red. (mg/kg)	Dose of Dithizon (mg/kg)	Blood glucose concentration (mM)				
			before	1st day	3rd day	6th day	9th day
1	985	49,3	5,9	6,7	6,2	4,3	5,8
2	1010	50,6	5,1	7,1	5,3	6,0	5,9
3	1012	52,2	4,3	6,1	5,7	6,2	6,8
4	1020	50,8	5,5	7,2	6,6	5,4	5,7

Table 3

Blood glucose concentration after injection of Glutathione Oxidized form and Dithizon

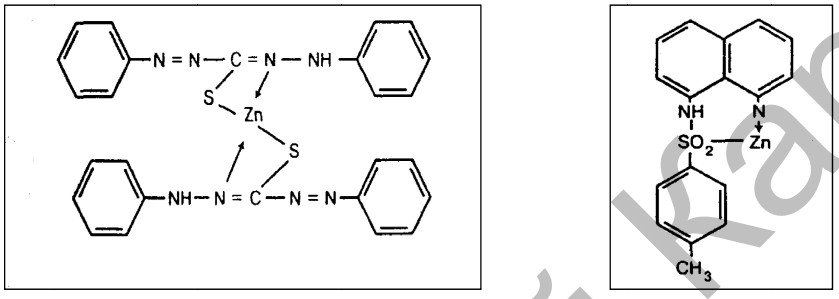
Animals	Dose of Gluthation Ox. (mg/kg)	Dose of Dithizon (mg/kg)	Blood glucose concentration (mM)				
			before	1st day	3rd day	6th day	9th day
1	960	47,2	8,9	15,7	12,5	14,3	15,8
2	1000	51,7	5,1	9,1	13,6	11,9	14,8
3	1020	50,1	4,3	12,5	10,6	14,2	21,8
4	1015	52,5	5,5	10,5	15,8	18,8	20,6

Discussion

Obtained results showed that administration of R-Glutathione result binding of almost all amount of Zn-ions in B-cells reversibly as least for 24 hours. Injection of dithizon after R-Glutathione not accompanied by forming in B-cells of chelat complexes Zn-DZ that result prevention of damage and death of majority B-cells and prevention developing of diabetes in 3 animals from 4. It is known that amino acids Cystein and L-Hystidine possess same property: injection of acid result protect B-cells of destruction by Dithizon as of developing of diabetes in animals [6]. However, administration of Oxidized Gluthatione not contains in structure of SH-radicals not protect B-cells of formation of Zn-DZ complex and of destruction of B-cells as of developing of diabetes [13]. Binding of Zn-ions of diabetes B-cells by a glutathione is apparently confirmed by existence of negative reaction for Zn for 24 hours. After that the complex gradually dissociated up and 48–72 hours later dithizon is able to form in B-cells toxic complex that accompanied by developing of experimental diabetes in animals.

It is known that in process of formation of the Zn^{+2}-complex with Dithizon and diabetogenic derivatives of 8-oxyquinolin atom of zinc is fixed between S or O atoms in position 8, and N or O atoms — in positions 1 or 2 (Fig. 2) [14]. Diabetogenic derivatives of 8-oxyquinolin have in the 8 position of quinolin ring active OH^- radical or other radicals contains atoms of S, N or O (Fig. 2). Isomers of 8-oxyqunolines not contains in this position such radicals or atoms, or if these radicals extracted from a molecule — not capable to form complex salts with zinc and not possess completely diabetogenic properties. It is necessary to return the active radicals in position 8 for to restore diabetogenic activity of substance [15]. Formation of the chelat complex by atoms of O and N accompanied by forming of pentagonal or hexagonal rings [14].

SH group contains sulfur atom. Meanwhile, as described above, it is known that sulfur atom participates in formation of the chelate complexes with Zn as well as N, O and C atoms. It is known that in process of formation of the Zn^{+2}-complex with Dithizon and diabetogenic derivatives of 8-oxyquinolin atom of zinc is fixed between S or O atoms in position 8, and N or O atoms — in positions 1 or 2 (Fig. 2) [14].



Dithizon 8-TSH

Figure 2. Chelat complexes Zn^{+2}–Dithizon and Zn^{+2}–8TSH

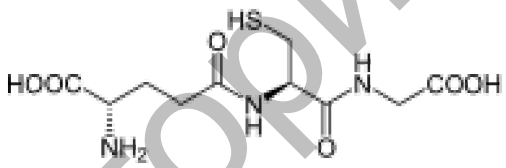


Figure 3. Chemical structure of R-Glutathione

On the base of obtained results we suppose that negative fluorescent reaction for Zn in B-cells after administration of Glutathione Reduced form determined by binding of Zn-ions via atom of sulfur of the SH-group and followed disposition of zinc atom between atom of sulfur and, probably, atom of oxygen or nitrogen (Fig. 3).

Conclusions

1. Amino acid Glutathione Reduced form contain in structure of SH-radical, 1000 mg/kg protect B-cells of formation complexes with diabetogenic zinc binding chelators that result prevention of destruction of cells prevention of developing of diabetes in animals.

2. Amino acid Glutathione Oxidized form not contain in structure of SH-radical, 1000 mg/kg not protect B-cells of formation complexes with diabetogenic zinc binding chelators that result destruction of cells and developing of diabetes in animals.

3. Administration of Glutathione Reduced form to animals result blocking of Zn-ions in B-cells that protect of interaction of metal with diabetogenic chelators.

4. Complex «Zn-Glutathione Oxidized form» in B-cells dissociated over 48 hours after forming.

5. We suppose that preventive effect after administration of Glutathione Reduced form determined by binding of Zn-ions via atom of sulfur of the SH-group and followed disposition of zinc atom between atom of sulfur and atom of oxygen or nitrogen.

References

1 Okamoto K. Experimental production of diabetes / K. Okamoto // Diabetes mellitus: Theory and Practice. — NY: McGraw-Hill Book company, 1970. — P. 230–255.

2 Kawanishi H. Secretion of B-granules in islets of Langerhans in association with intracellular reactive zinc after administration of Dithizone in rabbits / H. Kawanishi // Endocrinol. Jap. — 1966. — Vol. 13, No. 4. — P. 384–408.

3 Лазарис Я.А. К механизму повреждения панкреатических островков при дитизоновом диабете / Я.А. Лазарис, Г.Г. Мейрамов // Бюллетень экспериментальной биологии и медицины. — 1974. — № 3. — С. 19–22.

4 Мейрамов Г.Г. Ультраструктура панкреатических В-клеток при дитизоновом диабете и его предупреждении диэтилдитиокарбаматом натрия / Г.Г. Мейрамов, Н.И. Труханов // Проблемы эндокринологии. — 1975. — № 6. — С. 92–95.

5 Мейрамова А.Г. Диабетогенные цинксвязывающие В-цитотоксические соединения / А.Г. Мейрамова // Проблемы эндокринологии. — 2003. — Т. 49, № 2. — С. 8–16.

6 Meyramov G.G. Cystein Protect Pancreatic B-cells of Destruction Caused by Zn^{+2}-chelators / G.G. Meyramov, A.G. Meyramova // DIABETES, the Journal of American Diabetes Association, USA. — 2003. — Vol. 51, No. 6. — P. 552.

7 Kvistberg D. Staining of insulin with aldehyde fucshin / D. Kvistberg, G. Lester, A. Lasarov // J. Histochem Cytochem. — 1966. — Vol. 14. — P. 609–611.

8 Ortman R. Concerning the staining properties of aldehyde basic fucshin / R. Ortman, W. Forbes, A. Balasubramanian // J. Histochem. — 1966. — Vol. 14. — P. 104–111.

9 Orci G. Some aspects of the morphology of insulin secreting cells / G. Orci // Acta Histochem. — 1976. — No. 1. — P. 147–158.

10 Божевольной Е.А. 8-пара(толуолсульфониламино)хинолин — люминесцентный реактив на цинк и кадмий / Е.А. Божевольной, Г.В. Серебрякова // Химические реактивы и препараты. — М., 1961. — С. 36–42.

11 Красавин И.А. Гистохимические реакции на цинк в островках Лангерганса и диабетогенная активность используемых для этой цели реактивов / И.А. Красавин, З.Е. Бавельский, Я.А. Лазарис, В.М. Дзиомко // Проблемы эндокринологии. — 1969. — № 3. — С. 102–105.

12 Meyramov G.G. Histochemical and Immunocytochemical Investigation of Endocrine Tissue of Pancreas after Damage Caused by B-cytotoxic Chemicals and its Prevention by L-Hystidine / G.G. Meyramov // Bulletin of the Karaganda University. Biology, medicine, geography ser. — 2017. — No. 1(85). — P. 60–71.

13 Meyramov G.G., Meyramova A.G. Prevention of Diabetes Induced by Chelators by L-Hystidine / G.G.Meyramov, A.G.Meyramova // DIABETES, the Journal of American Diabetes Association, USA. — 2004. — Vol. 52, No. 6. — P. 583.

14 Альберт Э. Избирательная токсичность / Э. Альберт. — М., 1971. — 294 с.

15 Kotake Y., Kato T. Inhibitory action of etheral sulfate of xanturenic acid and kynurenic acid with regard to its diabetogenic property / Y. Kotake, T. Kato // Proc. Jap. Acad. — 1956. — Vol. 32. — P. 361–363.

Г.Г. Мейрамов, А.Ж. Шайбек, Г.Т. Картбаева, Е.М. Ларюшина,
Г.О. Жузбаева, А.Р. Алина, О.-Н. Дюпонт, С.С.Тыржанова

Қалпына келген глютатион көмегімен хелатор аркылы панкреатит В-жасушаларының бұзылуын болдырмаудың алдын алу

Авторлар мырышбайланыстырушы диабетогенді заттар тудыратын диабет дамуына глютатион аминқышқылының алдын алу әсерін зерттеген. Алдын алу әсер қасиетіне глютатионның тотыққан түріндегі формасы емес, құрылымында SH-радикалы бар глютатион қалыпты формасы ие екендігі көрсетілген. Глютатион қалыпты формасын жануарларға 1000 мг/кг дозасында бір ретті енгізу В-жасушаларының мырышпен байланысуына толығымен кедергі келтіріп, В-жасушаларының бұзылуын тудырып, жануарларда диабет тудыратындығы анықталды. Қалыпты глютатионның мырыш аралшығымен әрекеттесу химизмын зерттеу оны енгізгеннен кейін В-жасушаларында мырыштың толық бұғатталуы жоғары ерекше гистохимиялық әдістер көмегімен гистохимиялы түрде көрініс бермейді. В-жасушаларындағы мырыштың бұғатталу уақыты ұзақтығын зерттеу нәтижесі мырыш-глютатион кешені 36 сағ кейін мырыштың біртіндеп босап шығуымен ыдырай бастайды, ал 48 сағ соң кешен көп мөлшерде диссоциацияланады. Авторлар қалыпты глютатионның алдын алу бейімділігі оның аралшық мырыш пен байланысуын көрсетеді; мырыш атомы глютатион молекуласындағы SH-радикалы күкірт атомы мен оттегі не азот атомымен қосылып, диабетогенді хелатор енгізген есебінде танылады.

Кілт сөздер: В-жасушалар, R-глютатион, инсулин, мырыш, эксперименталды диабет.

Г.Г. Мейрамов, А.Ж. Шайбек, Г.Т. Картбаева, Е.М. Ларюшина,
Г.О. Жузбаева, А.Р. Алина, О.-Н. Дюпонт, С.С.Тыржанова

Предотвращение деструкции панкреатических В-клеток хелаторами с помощью восстановленной формы глютатиона

Авторами исследовалось предупреждающее влияние аминокислоты глютатиона в отношении развития диабета, вызываемого цинксвязывающими диабетогенными веществами. Показано, что предотвращающим действием обладает восстановленная форма глютатиона, содержащая в своей структуре SH-радикал, в отличие от окисленной формы глютатиона, не содержащей его. Установлено, что однократное введение восстановленного глютатиона животным в дозе около 1000 мг/кг полностью препятствует связыванию цинка В-клеток с формированием токсических комплексов, вызывающих разрушение В-клеток и развитие диабета у животных. Исследование химизма взаимодействия восстановленного глютатиона с островковым цинком показало, что введение его сопровождается полным блокированием цинка в В-клетках, в результате чего он не выявляется гистохимически с помощью высокоспецифичных гистохимических методов. Исследование длительности блокирования цинка в В-клетках показало, что комплекс цинк-глютатион начинает расщепляться с постепенным освобождением цинка через 36 ч, а через 48 ч комплекс в значительной степени диссоциирован. Авторы полагают, что предупреждающая способность восстановленного глютатиона обусловлена его способностью связывать островковый цинк; атом цинка при этом фиксируется между атомом серы SH-радикала и атомом кислорода или азота, содержащимися в молекуле глютатиона как и в случаях введения диабетогенных хелаторов, связывающих островковый цинк с помощью атома серы, с одной стороны, и либо атома кислорода, либо азота, или углерода — с другой.

Ключевые слова: В-клетки, R-глютатион, инсулин, цинк, экспериментальный диабет.

References

1 Okamoto, K. (1970). Experimental production of diabetes. *Diabetes mellitus: Theory and Practice*. NY: McGraw-Hill Book company.

2 Kawanishi, H. (1966). Secretion of B-granules in islets of Langerhans in association with intracellular reactive zinc after administration of Dithizone in rabbits. *Endocrinol. Jap. 13, 4,* 384–408.

3 Lazaris, Ya.A., & Meyramov, G.G. (1974). K mekhanizmu povrezhdeniia pankreaticheskikh ostrovkov pri ditizonovom diabete [On the mechanisms of destruction of pancreatic islets in diabetes caused by Dithizon]. *Biulleten eksperimentalnoi biolohii i meditsiny — Bull. Exp. Biol. Med., 3,* 19–22 [in Russian].

4 Meyramov, G.G., & Truhanov, N.I. (1975). Ultrastruktura pankreaticheskikh B-kletok pri ditizonovom diabete i eho preduprezhdenii dietilditiokarbamatom natriia [Ultrastructure of pancreatic B-cells in diabetes caused by Dithizone and its prevention by Diethyldithiocarbamic acid] *Problemy Endokrinolohii — Problems of Endocrinology, 6,* 92–95 [in Russian].

5 Meyramova, A.G. (2003). Diabetogennye tsinksviazyvaiushchie B-tsitotoksicheskie soedineniia [Diabetogenic zincbinding B-cytotoxic chemicals] *Problemy Endokrinolohii — Problems of Endocrinology, 49, 2,* 8–16 [in Russian].

6 Meyramov, G.G., & Meyramova, A.G. (2003). Cystein Protect Pancreatic B-cells of Destruction Caused by Zn^{+2}-chelators. *DIABETES, the Journal of American Diabetes Association, USA, 51, 6,* 552.

7 Kvistberg, D., Lester, G., & Lasarov A. (1966). Staining of insulin with aldehyde fucshin. *J. Histochem Cytochem., 14,* 609–611.

8 Ortman, R., Forbes, W., & Balasubramanian, A. (1966). Concerning the staining properties of aldehyde basic fucshin. *J. Histochem., 14,* 104–111.

9 Orci, G. (1976). Some aspects of the morphology of insulin secreting cells. *Acta Histochem., 1,* 147–158.

10 Bozhevolnoi, E.A., & Serebriakova, G.V. (1961). 8-para(toluolsulfonilamino)khinolin — liuminestsentnyi reaktiv na tsink i kadmii [8-para(toluenesulphonylamino)quinolin, a fluorescent reagent for zinc and cadmium] *Khimicheskie reaktivy i preparaty — Chemicals reagents and drugs*, Moscow, 36–42 [in Russian].

11 Krasavin, I.A., Bavelsky, Z.E., Lazaris, Ya.A., Dziomko, V.M. (1969). Gistokhimicheskie reaktsii na tsink v ostrovkakh Langergansa i diabetogennaia aktivnost ispolzuemykh dlia etoi tseli reaktivov [Histochemical reaction for zinc in islets of Langerhans and diabetogenic activity of reagents for this purpose]. *Problemy Endokrinolohii — Problems of Endocrinology, 3,* 102–105 [in Russian].

12 Meyramov, G.G. (2017). Histochemical and Immunocytochemical Investigation of Endocrine Tissue of Pancreas after Damage Caused by B-cytotoxic Chemicals and its Prevention by L-Hystidine. *Bulletin of the Karaganda University. Biology, medicine, geography ser., 1(85),* 60–71.

13 Meyramov, G.G., & Meyramova, A.G. (2004). Prevention of Diabetes Induced by Chelators by L-Hystidine. *DIABETES, the Journal of American Diabetes Association, USA, 52, 6,* 583.

14 Albert, A. (1968). *Izbiratelnaia toksichnost [Selective Toxicity]*. Moscow.

15 Kotake, Y., & Kato, T. (1956). Inhibitory action of etheral sulfate of xanturenic acid and kynurenic acid with regard to its diabetogenic property. *Proc. Jap. Acad., 32,* 361–363.