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On the mechanisms of prevention destruction of pancreatic B-cells induced by direct action of zinc binding chelators by reduced form of glutathione

It is known that reduced form of amino acid the glutathione (GRF), containing in structure of a molecule of SH-radical is capable to prevent developing of diabetes caused by group of diabetogenic zincbinding chemicals whereas the oxidized form of the glutathione (GOF) contrary to GRF only what does not contain in a molecule of SH-group, was completely incapable to prevent developing of diabetum caused by this group of substances. It was shown that treatment of animal GRF is followed by emergence the completely negative reaction to zinc B-cells that it is possible to explain with binding of zinc with GRF what interfered with its interaction with diabetogenic ligands. The model of the isolated pancreatic islets at which the direct influence of substances on B-cells excluding possible interactions of the studied substances in blood and tissues is provided was applied to obtaining more convincing proofs. Results demonstrate that direct influence of GRF on B-cells of the isolated pancreatic islets really leads to binding zinc of B-cells thanks to what its interaction with diabetogenic helator is prevented. At the same time results demonstrate that preventive action of GRF contrary to GOF is caused by existence in structure of its molecule SH-group through which is forming a complex of zinc with GRF that protect destruction of B-cells at subsequent influence of diabetogenic zincbinding chemicals.

Keywords: B-cells, R-glutathione, insulin, zinc, experimental diabetes, reduced form of glutathione, oxidized form of glutathione, dithizon, 8-para(toluenesulphonylamino)quinolin.

Abbreviations

GRF — Reduced form of Glutathione;
GOF — Oxidized form of Glutathione;
Zn — zinc;
DZ — Dithizon;
8PTSQ — 8-para(toluenesulphonylamino)quinolin;
8-ox — Diabetogenic derivatives of 8-oxyquinlin.

Background

It is known that Diphenylthiocarbazone (Dithizon) and some derivatives of 8-oxyquinolin (8-ox) are forming 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 type 1 of 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 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. [5]. 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, **7**]. It was reported also that injection of Glutathione Reduced form protect B-cells of destruction caused by DZ and 8PTSQ [8, 9]. Meanwhile using of animal model is difficult to obtain confirmation that preventive action of GRF is realized by binding of Zinc in B-cells and not possible to exclude inactivation of DZ as result of possible interaction between GRF and DZ in blood, outside of B-cells. Aim of investigation: to investigate possible direct preventive effect of amino acids GRF and GOF on B-cells excluding possible interaction between chelator and Gluthatione in the blood.

Methods

Materials. Purified Isolated Pancreatic Islets [PI] from 18 Rats 4–5 days old. PI were isolated using of 2 % solution of Collagenase (SERVA, FRG) 3 times for 3 min. Washing 3 times in cold Hanks solution, pH 7.38–7.44. Selection by using of Dextran gradient concentration solution; washing 3 times in cold Hanks solution and followed manual selection of islets.

Group of islets 1.: 176 PI: Incubation in medium 199+Dithizon 5.6 mg/100 ml (51 mg/l) for 10 min; *Group 2*: 180 PI: Incubation in medium 199+98 mg/100 ml (980 mg/l) of GRF for 20 min; *Group 3*: 215 PI: Incubation in medium 199+1020 mg/l of GRF for 20 min.+ cultivation in medium 199+Dithizon 5.6 mg/100 ml (51 mg/l) for 10 min; *Group 4*. 152 PI. Incubation in medium 199+95.6 mg/100 ml (956 mg/l) of GOF for 20 min; *Group 5*. 165 PI. 152 PI. Incubation in medium 199+97.2 mg/100 ml (972 mg/l) of GOF for 20 min + cultivation in medium 199+Dithizon 5.4 mg/100 ml (54 mg/l) for 10 min.

Fixation of islets in Bouin for 45 min. Embedding in paraffin. Part number of PI were used for preparing of frozen cryostate sections. Staining technologies: Aldehyde-fucshine method [10–12] 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 extracted from PI on spectrum of absorbance correspond for 530 nm [3] that correspond for pure complex. Sections were investigated using dark microscopy

Staining by Dithizon. Preparing of Dithizon solution: 30 mg of Dithizon, (SIGMA, USA) +10 ml. bidistillate+0.2 ml 25 % NH_4OH 10 min. mixing on tempera- ture $+70^0$ at Celsium. Solution was injected intravenously to Rabbits and to Mice 46–48.6 mg/kg. Intensity of staining of B-cells by Dithizon and by 8PTSQ was measured by histofluorimetric method [13]. Zinc content was calculated as not direct parameter K=AB1/AB2 where: AB1-intensity of staining of islets treated by GRF+DZ and by GOF+DZ; AB2 — intensity of staining of intact islets treated by DZ only (1.00) and by 8PTSQ only (1.0).

Staining by 0.4 % acetone solution of 8-para(toluenesulphonylamino)quinolin /8PTSQ/, a high specific fluorescent reagent was used for staining of Zn-ions in B-cells. 8PTSQ formed fluorescent green complexes with Zn^{+2}-ions visible using fluorescent microscopy [14–16].

Results

Group 1. Incubation of intact islets with Dithizon result binding of Zinc in B-cells that accompanied by formation of red granules of chelat complex «Zinc-DZ» (Fig. 1.1) in cytoplasm of all number of islets.

Group 2. Preliminary incubation of islets with GRF 98 mg/100 ml (980 mg/l) accompanied by negative fluorescent reaction for Zinc ions in B-cells that determined by complete not diabetogenic binding f Zinc in B-cells by high concentration of GRF (Fig. 1.2; Table).

Group 3. Preliminary incubation of islets with GRF 95 mg/100 ml (950 mg/l) and followed incubation with Dithizone solution not result binding of Zinc in B-cells by Dithizon determined by binding of Zinc this period on by GRF (Fig. 1.3).

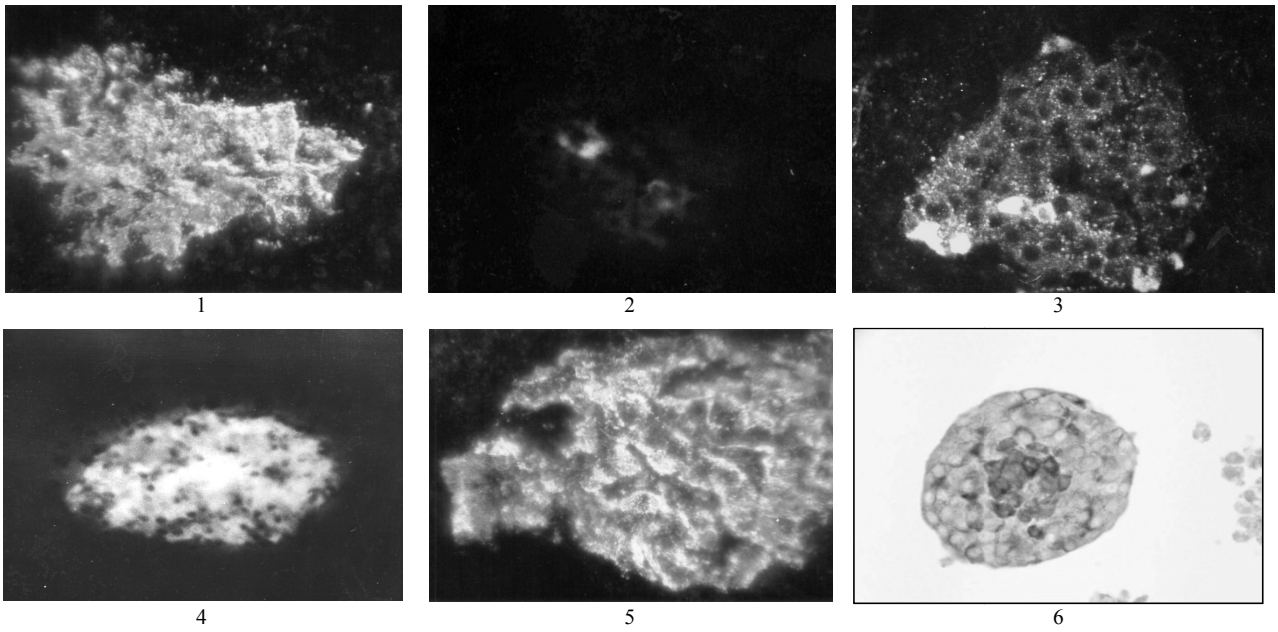
Group 4. Incubation in medium 199+95.6 mg/100 ml (956 mg/l) of GOF for 20 min; positive reaction for Incubation of islets with GOF, a oxidized form of Glutathione not contains in structure of molecule of SH-radical, not result binding of Zinc ions in B-cells that accompanied by intensive positive fluorescent reaction for Zinc ions in B-cells that determined by absence of ability of GOF to form complexes with Zinc in B-cells (Fig. 1.4).

Group 5. Incubation of islets with GOF, an oxidized form of Glutethione not contains in structure of molecule of SH-radical, not result binding of Zinc ions in B-cells that accompanied by followed formation of toxic complexes Zinc-Dithizone after past incubation with Dithizone solution (Fig. 1.5) and not protect B-cell of destruction (Fig. 1.6).

Obtained results showed that direct action of GRF on B-cells of isolated pancreatic islets result binding of almost all amount of Zn-ions in B-cells. Interaction with DZ after GRF not accompanied by forming in B-cells of chelat complexes Zn-DZ that result prevention of destruction and death of majority B-cells. Contrary, direct action of GOF not contains in structure of SH-radicals not protect B-cells of formation of Zn-DZ that accompanied by destruction and death of B-cells.

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) [15]. Diabetogenic derivatives of 8-oxyquinolin have in the 8 position of quinoline 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 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 [16]. Formation of the chelat complex by atoms of O and N accompanied by forming of pentagonal or hexagonal rings [6].



1 Isolated Pancreatic islet. Dithizon, Dithizon 5.6 mg/100 ml (51 mg/l). Large amount of red granules of complex Zn-DZ in B-cells; Dark microscopy; ×280.

2 Isolated Pancreatic islet. Incubation in medium 199+98 mg/100 ml (980 mg/l) of GRF for 20 min; negative fluorescent reaction for Zinc ions with 8PTSQ as result of blocking of Zinc by GRF; fluorescent microscopy; ×140.

3 Isolated Pancreatic islet. Preliminary incubation of islets with GRF 95 mg/100 ml (950 mg/l) and followed staining by Dithizon solution; absence of red granules of Zn-DZ complex in B-cells as result of preventive blocking of Zn by GRF; Dark micrscopy; ×140.

4 Isolated Pancreatic islet. Incubation in medium 199+95.6 mg/100 ml (956 mg/l) of GOF for 20 min; positive fluorescent reaction for Zinc with 8PTSQ; fluorescent microscopy; ×140.

5 Isolated Pancreatic islet of intact rabbit. Incubation in medium 199+97.2 mg/100 ml (972 mg/l) of GOF for 20 min + + cultivation in medium 199+Dithizon 5.4 mg/100 ml (54 mg/l) for 10 min. Positive reaction for Zinc: a large amount of red granules of complex Zn-DZ in B-cells; Dark microscopy; ×280.

6 Isolated Pancreatic islet of intact rabbit. Incubation in medium 199+97.2 mg/100 ml (972 mg/l) of GOF for 20 min + + cultivation in medium 199+Dithizon 5.4 mg/100 ml (54 mg/l) for 10 min+ incubation in fresh medium 199 fr 48h; aldehyde-fucshine staining: destruction of B-cells and negative reaction for insulin in B-cells; ×280.

Figure 1

Commentaries and conclusions for Figure 1

Obtained results showed that: 1) interaction of GRF with zinc in B-cells result forming of complex zinc-GRF (Fig. 1.2); 2) this complex is more stronger and completely protect interaction of zinc with Dithizon as of destruction oaf B-cells caused by complex zinc-Dithizon; 3) GOF on the contrary, does not interact with zinc and does not prevent its binding by Dithizon that result followed destruction of B-cells (Figs. 1.5, 1.6).

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) [6]. High durability of complex zinc-Dithizon determined by fixation of atom of zinc between two atoms of sulfur from two molecules of Dithizon [5] (Fig. 2). It is apparent that atom of zinc can be fixed between atoms sulfur and

oxygenium or atom of hydrogenium (Fig. 3) of the molecule of GRF; or between of 2 atoms of sulfur from the two molecule of GRF.

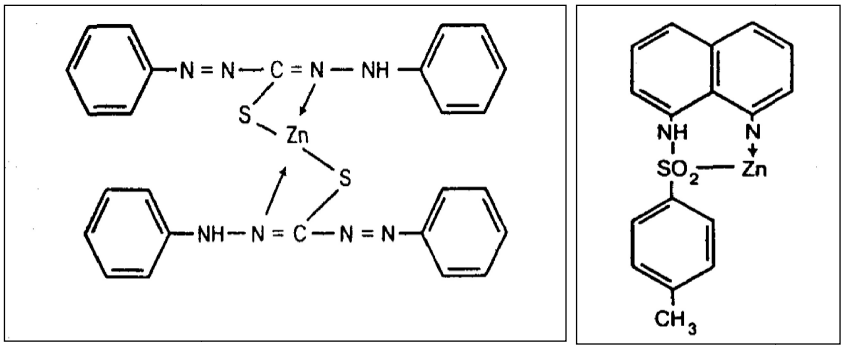
T a b l e

Intensity reaction for Zinc ions in B-cells of islets treated by DZ, GRF, and GOF [in relative units (ru)]

№	Experimental conditions	Histochemical methods for staining of Zinc ions	
		Dithizon-method (DZ)	Fluorescent 8PTSQ-method
1	Intact islets	1.00±0.01	1.00±0.02
2	GRF	0.03±0.01	0.04±0.01*
3	GRF + DZ	0.07±0.01*	0.03±0.01
4	GOF	0.97±0.03*	0.98±0.01*
5	GOF + DZ	0.95±0.03*	–

Note: * — $p \leq 0.001$.

Results obtained by using of two high specific for zinc histochemical methods showed that: 1) GRF formed with zinc in B-cells of not diabetogenic chelat complex (negative reaction for zinc in B-cells) and of followed binding of zinc by Dithizon that confirmed by negative reaction with Dithizon; 2) GOF not reacted with zinc in B-cells and not protect B-cells of interaction with Dithizon accompanied by formation of complex zinc-Dithizon.



Dithizon 8-PTSQ

Figure 2. Chelat complexes «Zn^{+2}-Dithizon» and «Zn^{+2} – 8PTSQ»

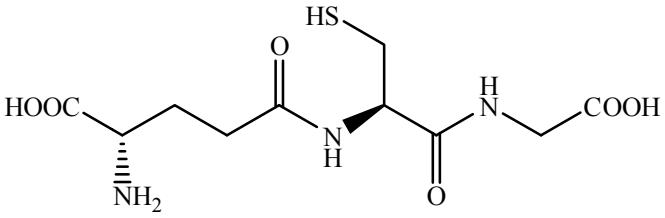


Fig.3. Chemical structure of Glutathione Reduced form

Analysis structure of molecule of GRF demonstrate obviously that atom of zinc can be fixed between atoms of sulfur and atoms of oxygenium or hydrogenium at process of formation of chelat compex zinc-GRF.

Conclusions

1. Amino acid Glutathione Reduced form contain in structure of SH-radical, 98 mg/100 ml (980 mg/l) result binding of zinc ions in cytoplasm of B-cells that protect B-cells of formation complexes with diabetogenic zinc binding chelators and of destruction of cells.

2. Amino acid Glutathione Oxidized form not contain in structure of SH-radical, 97.2 mg/100 ml (972 mg/l) not result binding of zinc ions in cytoplasm of B-cells that not protect B-cells of formation complexes with diabetogenic zinc binding chelators and of destruction of cells.

3. Ppreventive effect after direct action 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.

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Диабетогенді мырышбайланыстырушы заттардың тікелей әсерімен туындаған панкреатит В-жасушаларының деструкциясына жол бермейтін тотықсыздандырылған күйдегі глютатионның әсер ету механизмдері туралы

Молекула құрылымында SH-тобы бар қалпына келтірілген глютатион (GRF) аминқышқылы, диабетогенді мырышбайланыстырушы заттар туындататын диабеттің дамуын болдырмауға бейімді. Глютатионның тотыққан формасы (GOF), GRF формадан тек молекуласында SH-тобы жоқ болуымен ерекшеленеді және диабет дамуын толық тоқтата алмайды. Жануарларға енгізілген GRF форма В-жасушаларындағы мырышқа толық кері әсер етуі құбылысы байқалады, яғни бұл GRF мырышпен байланысы диабетогенді лигандпен әрекеттесуіне кедергі келтіреді деп түсіндіруге болады. Неғұрлым нақты дәлелдеме алу үшін зерттелінетін затың қан және басқа ұлпаларға енуін болдырмау үшін оқшауланған панкреатит аралшығы модель ретінде пайдаланылды, яғни заттар тікелей В-жасушаларына әсер етілді. Зерттеу нәтижелері оқшауланған панкреатит аралшықтарының В-жасушаларына GRF тікелей әсері мырышпен байланысын арттырады, соның әрекетінде диабетогенді хелаторлар

өзара әрекеттесуі тоқтайды. Сонымен қатар зерттеу нәтижесі, GRF мырыш жиынтығымен превентивті әрекеті, GOF қарсылығы молекула құрылымында SH-тобы болуымен түсіндіріледі, яғни GRF мырыш жиынтығының қалыптасуы диабетогенді мырышбайланыстырушы заттардың әсерінен В-жасушалардың бұзылуына жол бермейді деп болжанады.

Кілт сөздер: В-жасушалар, R-глютатион, инсулин, мырыш, эксперименталды диабет, қалпына келтірілген глютатион, глютатионның тотыққан формасы, дитизон, 8-пара(толуолсульфониламино)хинолин.

Г.Г. Мейрамов, А.Ж. Шайбек

О механизмах действия восстановленной формы глютатиона, предупреждающего деструкцию панкреатических В-клеток, вызванную прямым воздействием диабетогенных цинксвязывающих веществ

Известно, что восстановленная форма аминокислоты глютатион (GRF), содержащая в структуре молекулы SH-группы, способна предотвращать развитие диабета, вызываемого группой диабетогенных цинксвязывающих веществ, тогда как окисленная форма глютатиона (GOF), отличающаяся от GRF только тем, что не содержит в молекуле SH-групп, была полностью неспособна предотвращать развитие диабета, вызываемого этой группой веществ. Было показано, что введение животным GRF сопровождается появлением полностью отрицательной реакции на цинк в В-клетках, что можно объяснить связыванием цинка с GRF, что препятствовало его взаимодействию с диабетогенными лигандами. Для получения более убедительных доказательств была применена модель изолированных панкреатических островков, при которой обеспечивается прямое воздействие веществ на В-клетки, исключающее возможные взаимодействия исследуемых веществ в крови и тканях. Результаты свидетельствуют о том, что прямое воздействие GRF на В-клетки изолированных панкреатических островков действительно ведет к связыванию островкового цинка, благодаря чему предотвращается его взаимодействие с диабетогенными хелаторами. Одновременно результаты свидетельствуют о том, что превентивное действие GRF в противоположность GOF обусловлено наличием в структуре его молекулы SH-групп, через которые происходит формирование комплекса цинка с GRF, препятствующего разрушению В-клеток при последующем воздействии диабетогенных цинксвязывающих веществ.

Ключевые слова: В-клетки, R-глютатион, инсулин, цинк, экспериментальный диабет, восстановленная форма глютатиона, окисленная форма глютатиона, дитизон, 8-пара(толуолсульфониламино)хинолин.

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