ABSTRACT

Prevention by restored form of glutathione of the destruction of pancreatic β-cells caused by diabetogenic zinc binding chemicals and investigation of the mechanisms of its by preventive activity

Relevancy of the problem. In the last 40-50 years, there has been an intensive grooving of number of patients with diabetes mellitus in most countries. In Kazakhstan, the number of registered patients is also growing rapidly. In 1998, there were 93,000 of them, by the end of 2011 the number of patients increased to 208,000, and by 2016 it was close to 300,000. Over 18 years, the number of registered patients increased, thus, more than 3 times. The total number of patients, taking into account unregistered persons and persons with latent forms of the disease, is estimated at 600-700 thousands, which is about 3% of the total population of Kazakhstan compared to 0.7-0.8% in 1998. Most of 80- 90% are patients with type 2 diabetes. Every 10-12 years, according to the International Diabetes Federation, the number of people with diabetes in the world doubles. In developed countries of Europe, the number of patients is on average 5 to 8% of the total population, which is higher than in Kazakhstan, and in some countries it reaches 18-20%. More than 70 years ago, it was first established that certain chemical compounds are capable to induce a selective destruction and death of pancreatic β -cells, which is accompanied by their death and the development of diabetes mellitus. Today more than 20 substances with similar properties are known. The use of chemical models of diabetes mellitus in diabetology made it possible to obtain a large amount of data that made it possible to study the causes and mechanisms of the development of diabetes mellitus, since such models are "pure", that is, only the β -cells of the pancreas are damaged, causing an essential form of diabetes not associated with causally with other organs and systems of the body.

It has been established that among of more than 20 diabetogenic che- micals known to date, 18 are zinc-binding (chelators). Some of them are included as the main component in the composition of some antimicrobial drugs, or are synthesized in the body under certain conditions. Their administration into the body is accompanied within a few minutes by the binding of β-cells with zinc with the formation of toxic zinc-chelator complexes, which have a destru- ctive effect on B cells, leading to their death within a few minutes.

At present, the ability of certain substances, upon preliminary administration into the body, has been established to prevent the action of diabetogenic zinc-binding compounds during their subsequent administration and, thus, to prevent the development of diabetes. There are indications that some compounds containing SH-groups (sulfhydryl radicals) in the structure of the molecule, in particular, the amino acid glutathione, have this ability. The present study is focused on the study of the ability of one of these substances -the amino acid glutathione - to prevent the development of diabetes caused by zinc-binding chelators and to study the mechanisms of its protective action.

The purpose of the dissertation: to study the mechanisms of action of reduced glutathione, which prevents the development of experimental diabetes caused by diabetogenic zinc-binding compounds

The objects of investigation were insulin-producing β -cells of the pancreatic tissue and cultures of isolated pancreatic islets of white rats and rabbits, peripheral blood of animals with experimental dithizone diabetes and diabetes caused by 8-para (toluenesulfonylamino) quinoline (8TSQ).

Scientific novelty of the research.

1. For the first time, the ability and mechanisms of the amino acid reduced glutathione to prevent the development of experimental diabetes caused by diabetogenic zinc-binding compounds were investigated. It has been shown that this ability is due to the presence of an SH-radical in the reduced glutathione molecule, due to which zinc is blocked, which prevents the formation of chemical complexes with chelators that destroy β-cells.

2**.** This work is the first investigation related to the study of the mechanisms of the antidiabetic action of glutathione as well as amino acids containing the SH-radical. This is also the first study in which highly specific and sensitive methods of histochemical and histological analysis of the tissue of the pancreas and pancreatic islet β-cells were used, as well as the method of culture of isolated pancreatic islets, which makes it possible to study the nature of the direct effect of diabetogenic substances and amino acids containing the SH-radical. directly on β -cells and excludes possible extrapan creatic effects.

The practical significance is due to the fact that, in contrast to the previously known methods of preventing diabetes caused by DCV, using chemicals that are not contained in the body and are not involved in metabolic processes, the amino acid glutathione constantly enters the body, participating in various metabolic processes, which is potentially significant increases interest in it in terms of possible use to prevent the development of diabetes associated with the action of DCV, in particular, those of them that are endogenously synthesized in the body with some metabolic disorders or enter the body from the outside, including when using certain pharmaceuticals with antimi-crobial action, the main whose components are diabetogenic derivatives of 8-hydroxyquinoline, which are among the most active zinc-binding diabetogenic substances

The structure and volume of the thesis. The structure of the thesis is determined by the tasks and consists of definitions, symbols and abbreviations, introduction, review of literature, material and methods, results and discussion, conclusion, list of used literature sources. It is set out on 128 pages of typew- ritten text, illustrated with 19 tables and figures, contains a list of references from 197 titles.

Main results.

1.It was established for the first time that the amino acid as reduced glutathione, containing SH-groups in its structure, at a dose of 1000 mg/kg is able to prevent the development of experimental diabetes in all experimental animals caused by diabetogenic zinc-binding β-cytotoxic compounds dithizone and 8-TSQ,

while oxidized glutathione, which differs from the reduced one by the absence of SH-groups in its structure, does not prevent its occurrence in animals

2.It was shown for the first time too that in the mechanism of action of glutathione, like the amino acids of cysteine, its ability to bind islet zinc due to its interaction with the SH-radical (sulfhydryl groups) is of primary importance, preventing DZC (Diabetogenic Zinc binding Chemicals) from forming toxic complexes with zinc that destroy β -cells. It was established for the first time that oxidized glutathione, which has the same chemical structure, but does not contain the SH-radical, does not have an antidiabetic effect

3.In experiments on the culture of isolated pancreatic islets using two forms of glutathione, it was first established that reduced glutathione has not an indirect extrapancreatic, but a direct effect directly on β -cells, which is accompanied by almost complete binding of islet zinc, which prevents the action of zinc-binding diabetogenic substances.

4.It has been shown for the first time that the using of reduced glutathione is accompanied by an increase in the level of activity of antioxidant enzymes of the glutathione link system, which takes an active part in neutralizing toxic compounds entering or forming in the body, additionally providing its protective properties in relation to DZC.

5.It has been shown for the first time too that the method of suppressing endogenous synthesis in the body of one of the zinc-binding substances -4,8-dihydroxyquinoline-2-carboxylic acid (xanthurenic acid) -is less effective: its level in urine, although it was reduced by 3 times compared with the experimental animals, but still remained 2.5-3 times higher than in the control group, which contributed to a decrease in the level of gleemia from 12.4 mmol / l to 8.0 mmol / l.

Scientific and practical significance of the work.

The theoretical significance of the study lies in the fact that experimental data were obtained showing the ability of the amino acid reduced glutathione to prevent the development of diabetes mellitus caused by a wide group of diabetogenic zinc-binding substances (DZC). It was found that the preventive effect of glutathione is due to its ability to form intracomplex compounds with zinc contained in pancreatic β-cells, which prevents its interaction with diabetogenic zinc-binding substances during their subsequent administration. Direct evidence has been presented that blocking zinc with glutathione is due to the presence of sulfhydryl SH-groups in the structure of the glutathione molecule, the sulfur atom of which has a high chemical affinity for zinc. It has also been established that glutathione increases the acti vity of antioxidant enzymes, which additionally helps to reduce the toxic effect of zinc-binding substances.

Approbation of work and publications

The main results presented in the dissertation are reflected in 28 publications, including 3 articles in cited foreign journals on the Thomson&Reuters and Scopus databases, in 12 articles recommended by the KKSON of the Ministry of Education and Science in 3 articles in journals based on the RINC. 3 works were presented and published in the materials of European and American scientific

congresses. A number of works were also presented and published in the materials of international conferences held in Kazakhstan and Russia.

The main results were discussed at the 75th American Congress of Diabetologists (Boston, 2015), at the 18th European Congress of Transplantology (Barcelona,2017), at the European Congress on Eyes Complicatins in Diabetes (France, 2014), at the European Congress on Technologies of Treatment of Diabetes (Vienna, 2018) and at the 22nd World Diabetes Congress (Melbourne, 2013), and at the 15th Congress of the International Association of Morphologists (Khanty-Mansiysk, Russia, 2020) Based on the results of the research, 1 methodological manual, Acts of the implementation of the research results in the practice of scientific work and in the educational process at the universities of Astana and Khanty-Mansiysk were received.