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Investigation of Antidiabetogenic Effect of the Iodine-Selenium Concentrate in Animals with Chronic Alloxan Diabetes of Varying Severity

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Abstract

BACKGROUND: The diabetogenic effect of alloxan is known and is determined by ability to stimulate lipid peroxidation processes in B-cells of the pancreas.

AIM: This study aims to investigate of the possible antidiabetic action of long time prolonged of iodine-selenium concentrate action in rats with alloxan diabetes.

METHODS: Reproduction of experimental alloxan diabetes was carried out in rats by a single intravenous injection of alloxan 35–43 mg/kg body weight. The "iodine-selenium" concentrate was administered per OS through a tube at the rate of 1.25 ml/100 g of the concentrate. In experimental animals with mild and heavy diabetes mellitus, the level of glucose in the blood was assessed, products of LPO-AOD; the state of the histostructure of the pancreas and of insulin content in B-cells were studied using of aldehyde fuchsin and diethylpseudoisocyanine methods.

RESULTS: Long time prolonged administration of the "iodine-selenium" concentrate (60 days) to animals with mild experimental diabetes mellitus is accompanied by a significant decrease in blood glucose levels by 1.89 times compared to the control ($p < 0.05$) and of LPO within normal values as by increase of the level of glutathione peroxidase (GPO) by 2.23 times compared with the initial ($p < 0.01$), by prevention of the development of histological changes in pancreatic islets and a slight decrease of insulin content in B-cells. Under similar experimental conditions in animals with severe alloxan diabetes, the level of glycemia significantly decreased from 20.23 ± 2.15 mmol/l to 12.39 ± 1.52 mmol/l as of the level of diene conjugates of erythrocytes and plasma, as decrease of ketodienes, MDA of plasma and in erythrocytes and primary lipid peroxidation products, while remaining elevated, despite an increase in GPO by 50.0% compared with control ($p < 0.05$) in the presence of histological changes in the pancreatic islets as in experimental diabetes.

CONCLUSIONS: The antidiabetic effect of the "iodine-selenium" concentrate in rats with mild alloxan diabetes on the level of glycemia, LPO–AOD and state of the histostructure of the pancreas and the content of deposited insulin in pancreatic B-cells, is probably due to antioxidant effect of selenium to stimulate activity of glutathione blocking lipid peroxide and hydrogen peroxide in alloxan diabetes mellitus.

Introduction

The search for effective methods of correction of metabolic disorders in diabetes mellitus is one of the actual problems. Despite number scientific publications today, there is no clear to understand the mechanisms of diabetogenic effect of alloxan. It is known that alloxan, causing the generation of free radical oxidation processes, plays a role in the pathogenesis of diabetes mellitus [1], [2], [3], [4], [5], along with its possible effect on B-cells similar to zinc-binding chemicals, which cannot be excluded [6], [7], [8], [9], especially considering the rate of selective destruction of B-cells by alloxan, which is as marked as in diabetes caused by diabetogenic zinc-binding chemicals, as well as the presence of a negative reaction to zinc in B-cells not only after a few days but also after a short time after its injection. It was established that selenium is necessary for the synthesis of one of the enzymes of the glutathione link of the antioxidant defense system glutathione peroxidase (GPO) [10], [11]. The action of the aqueous concentrate of iodine compounds, unlike others, is that in the "iodine-water" system associates are formed by weak hydrogen bonds, that is, iodine ions in water are connected not with hydrogen but with oxygen that result prevention of overdose and side effects of "iodine concentrate" [12], [13]. The influence of these microelements was investigated in various epidemiological and experimental studies in diabetes mellitus, however, insufficient attention was paid to the analysis of the chemical mechanisms of interaction to understand the mechanism of their anti-diabetogenic effect [14].

The aim of the study is to investigate the possibility of the antidiabetic effect of the

iodine-selenium concentrate after administration to rats with experimental diabetes for dynamics of changes in carbohydrate metabolism, the level of lipid peroxidation products and of activity of antioxidant enzymes as the state of the histostructure and content of deposited insulin in pancreatic B-cells.

Materials and Methods

In a chronic experiment, 60 August and Wistar rats were used (m = 170–210 g) contained in a standard diet. In all animals, experimental alloxan diabetes was induced by intravenous injection of 4% water solution of alloxan 35–43 mg/kg body weight. Ten–14 days after injection, 24 animals were selected of which two groups were isolated (n = 16). Group 1 with a mild course of diabetes (n = 8) in which the level of glycemia did not exceed 12 mmol/l and Group 2 with heavy and moderate diabetes, with a glycemia level above 12 mmol/l (n = 8). To avoid the development of ketoacidosis, animals were injected with insulin at the rate of 0.5 units/100 g of body weight. During experience, the dose of insulin was reduced by 13–30 units/100 g [15].

The iodine-selenium concentrate was introduced per OS at the rate of 1.25 ml/100 g of the concentrate dissolved in 5 ml of water. For to estimate a metabolic parameters in experimental animals, their tail veins were drawn from the blood. Blood glucose was measured using of glucose peroxidase method. The intensity of LPO processes was evaluated in plasma by the level of diene conjugates (DC) and MDA; in erythrocytes, according to the level of DC, ketodienes (KD), malondialdehyde (MDA), the total number of primary (SPP), and secondary (SVP) products [16], [17], [18], [19], [20]. The antioxidant status of erythrocytes was evaluated by the activity of superoxide dismutase (SOD), catalase, and GPO) [21], [22], [23], [24].

The concentration of primary lipid peroxidation products in the blood was determined by measuring the ultraviolet absorption of lipid extracts in the range from λ = 232 to 268 nm. Secondary lipid peroxidation products were evaluated by the level of TBA-active products in reaction with thiobarbituric acid. The content of SPP and SVP was calculated by the absorption ratio at λ = 232 nm in the first case and E268 and E220 in the second. SOD activity was measured by the reaction of reduction of Phormasan from tetrazolium salts in a slightly alkaline medium. The method for to estimate the activity of catalase is based on the ability of hydrogen peroxide to form a stable colored complex with molybdenum salts, the color intensity at λ = 410 nm. The activity of erythrocyte GPO was evaluated spectrophotometrically by the accumulation of oxidized glutathione. The state of the pancreatic histostructure and of insulin content in B-cells were evaluated using aldehyde fuchsin and insulin-specific diethylpseudoisocyanine methods [25], [26] using a histofluorimetric complex [27]. Digital processing of the materials was carried out using standard Microsoft Excel programs. The research results were processed statistically with the calculation of average indicators and the error of the mean (M ± m); the significance of differences between the compared values was evaluated using the parametric Student's t-test. The preliminary data obtained in the study were checked for normal distribution and for equality of the general variances of the compared populations, and it was found that they meet these requirements. The differences of results of the study at $p < 0.05$ were valid. The correlation dependence was calculated using Pearson correlation analysis.

Results

Administration of the iodine-selenium concentrate to experimental animals of Group 1 for 60 days results a significant decrease in plasma of glucose levels for 1.89 times in compared with control ($p < 0.05$) to a level close to the initial one. A similar trend was observed in terms of the level of lipid peroxidation products (Table 1) and the rate of decrease in lipid peroxidation in erythrocytes was higher than in blood plasma by 12.6% (DC) and 14.0% (MDA).

The analysis of AOZ enzyme indices revealed more significant changes from the HPO side, the level of which in experimental rats of the Group 1 increased after the 60-day experiment by 2.23 times in compared with the initial one ($p < 0.01$) while the content of SOD and catalase increased by 20.0% and 16.0%, respectively, comparatively with control (Table 2).

An obvious distinct direct correlation was established between the blood glucose level and DC and MDA values in blood plasma ($r = 0.8963$; $p < 0.05$), while between the glucose levels and these toxic derivatives of lipid peroxidation in erythrocytes ($r = 0.8942$ and $r = 0.8371$, respectively; $p < 0.05$).

Marked feedback is noted ($r = -0.8954$; $p < 0.05$) as a result of calculation of correlation coefficient between the level of glucose and GP. Long-term administration of iodine-selenium concentrate to experimental animals of Group 2 accompanied by decrease in blood glucose level by 38.8% in compared to control values ($p < 0.05$). In experimental animals, a significant decrease in the primary lipid peroxidation products in blood erythrocytes was noted: DK by 40.5% and SPP by 38.2%, while the level of SVP remained practically unchanged. The rate of decrease of toxic derivatives of lipid peroxidation (DK, MDA) in blood plasma in experimental animals was higher, in contrast to erythrocytes by 9.9% (DK) and 13.5% (MDA), respectively. On the part of AOD enzymes, it was noted

a significant increase of the level of HPO by 50.0% in compared with control ($p < 0.05$), while the activity of SOD and catalase did not exceed 13.4% and 7.1%. A strong direct correlation is observed between the level of glucose and products of CPO: DC and MDA both in blood plasma ($r = 0.8542$ and $r = 0.8633$, respectively; $p < 0.05$) and in erythrocytes ($r = 0.8941$ and $r = 0.8235$, respectively; $p < 0.05$), while the inverse correlation is observed between the level of glucose and GPO in the blood ($r = -0.8124$; $p < 0.05$).

Thus, obtained results showed that prolonged administration of iodine-selenium concentrate to rats with mild experimental diabetes mellitus is accompanied by a preventive effect, clearly confirmed by the results of histological examination of pancreatic tissue and by content of insulin and zinc in B-cells. It was shown that in experimental animals, the histostructure of islets retained its usual structure with a slight decrease in the insulin content in B-cells (Figure 1 and Table 3) which was not clearly detected by visual examination.

Discussion

Diabetes, first discovered using of alloxan (Dunn *et al*. 1943) [28], is one of the first models of experimental diabetes induced by selective destruction of B-cells. Meanwhile, the mechanisms of the damaging effect of alloxan on B-cells are not cleared today. Before alloxan, in 1942 [29], a model of diabetes caused by dithizone was obtained, the mechanisms of the damaging action of which are well understood. It was found that the administration of dithizone to animals is accompanied by a sharp increase in the content of DC and thiobarbituric acid in the blood and erythrocytes, which increases the toxicity of dithizone to B-cells.

It is known that alloxan diabetes contributes to the development of disorders in carbohydrate metabolism, generates free radical oxidation processes in the blood, due to the duration and severity of the disease, is accompanied by a breakdown of the dynamic balance between the accumulation of lipid peroxides and the state of antioxidant protection [1], [4], [30], [31]; accompanied by an inductive increase in the activity of the key AOD enzyme – SOD as slight increase of activity of catalase with a simultaneous inhibition of the glutathione-dependent AOD [32], [33], [34]. Therefore, LPO processes play an important role in the course of alloxan diabetes, which aggravates the state of experimental animals. Analysis of state of histostructure of the islets of animals with mild and heavy experimental diabetes showed the presence of dystrophic changes, an increase in the number of new formed B-cells in the preserved islets and a slight increase of the intensity of fluorescence of B-cells correspond to increase of deposited insulin in B-cells. The morphological changes of pancreas in alloxan diabetes correspond

Table 1: Concentration of glucose and lipid peroxidation products in rat blood after prolonged administration of iodine-selenium concentrate (1.25 ml/100 g) with alloxan diabetes (M ± m)

Indicators	Experience conditions				
	Intact	Mild form of diabetes		Heavy form of diabetes	
		Alloxan	Alloxan + «iodine-selenium»	Alloxan	Alloxan + "iodine-selenium"
Blood glucose concentration, mmol/l	4.6 ± 0.20	11.16 ± 2.03	5.90 ± 0.11*	20.23 ± 2.15	12.39 ± 1.52*
DC of blood plasma, nmol/ml	0.69 ± 0.10	1.33 ± 0.037	0.70 ± 0.019**	2.02 ± 0.71	1.00 ± 0.35*
DC erythrocytes conventional units	25.97 ± 0.82	67.88 ± 2.49	27.15 ± 0.99**	119.80 ± 3.88	71.28 ± 2.31*
KD erythrocytes conventional units	7.98 ± 0.69	15.28 ± 1.96	8.01 ± 0.12*	30.87 ± 2.36	21.61 ± 1.65
SPP erythrocytes conventional units	0.144 ± 0.028	0.266 ± 0.055	0.156 ± 0.032**	0.505 ± 0.017	0.312 ± 0.011*
SPP erythrocytes conventional units	0.127 ± 0.038	0.220 ± 0.050	0.131 ± 0.029*	0.387 ± 0.024	0.325 ± 0.015
MDA, plasma, nmol/ml	3.32 ± 0.07	6.83 ± 0.22	3.69 ± 0.11**	10.20 ± 1.65	6.89 ± 1.55*
MDA, mkm (ml) erythrocytes	6.63 ± 0.16	17.98 ± 1.45	7.19 ± 0.58**	31.62 ± 2.55	25.61 ± 1.85

*-**p < 0.05–0.01 – significance of differences compared to control. DC: Diene conjugates, MDA: Malondialdehyde, KD: Ketodienes

Table 2: Effect of prolonged administration of "iodine-selenium" on blood glucose concentration and activity of the enzymatic part of AOD in blood erythrocytes with alloxan diabetes (M ± m)

Indicators	Experience conditions				
	Intact	Mild form of diabetes		Heavy form of diabetes	
		Control	"Iodine-selenium"	Control	"Iodine-selenium"
Blood glucose mmol/l	4.6 ± 0.20	11.16 ± 2.03	5.90 ± 0.11*	20.23 ± 2.15	12.39 ± 1.52*
GPO erythrocytes un.act./ml	96.9 ± 2.57	74.61 ± 2.03	166.38 ± 4.53**	76.35 ± 1.96	114.53 ± 2.94*
SOD erythrocytes conventional un.act./ml/min	24.25 ± 0.14	21.13 ± 0.16	25.36 ± 0.19	28.01 ± 1.85	31.76 ± 2.09
Catalase erythrocytes conventional un.act./ml/min	0.33 ± 0.06	0.25 ± 0.05	0.29 ± 0.06	0.28 ± 0.017	0.30 ± 0.018

*-**p < 0.05–0.01 – significance of differences compared to control. GPO: Glutathione peroxidase

Table 3: Insulin and zinc content in B-cells of pancreatic islets (in relative units, r.u.) (M ± m)

No.	Method of staining	Insulin and zinc content in B-cells of pancreatic islets (in relative units, r.u.)		
		Control (intact)	Group 1 (mild form of diabetes)	Group 2 (heavy form of diabetes)
1	Aldehyde-fuchsin (insulin)	1.00 ± 0.11 (n = 16)	0.78 ± 0.09 (n = 22); mild form of diabetes; 0.57 ± 0.08 (moderate form of diabetes	*0.22 ± 0.05 (n = 31); destruction of majority of B-cells, marked decreasing of insulin content in cells
3	Diethylpseudoisocyanine (insulin)	1.00 ± 0.06 (n = 17)	0.82 ± 0.06 (n = 28)	**0.17 ± 0.03 (n = 30), marked decreasing of insulin content in B-cells
5	Para(toluenesulfonylamino)quinoline (8TSQ) (zinc)	1.00 ± 0.07 (n = 22)	0.77 ± 0.11(n = 22)	***0.08 ± 0.02 (n = 23), marked decreasing of zinc content in B-cells

*-**-***p < 0.005 – significance of differences compared to control

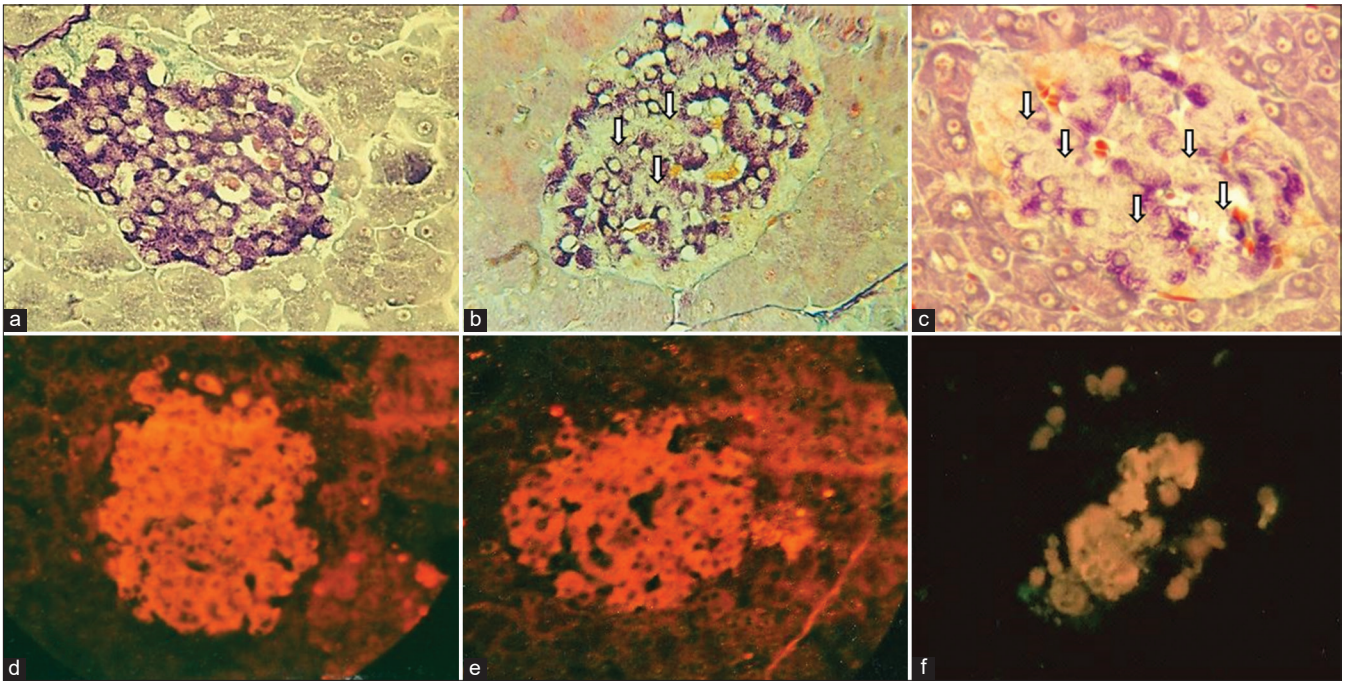

Figure 1: Histostructure and content of insulin and zinc in pancreatic B-cells of pancreatic islets of intact and experimental animals. (a) Intact islet, aldehyde fuchsin; histostructure unchanged; the usual normal amount of insulin in B-cells (violet color); ×280; (b) mild form of diabetes, aldehyde fuchsin; signs of damage of single B-cells (left side in the center), a slight decrease in insulin content; ×280; (c) heavy form of diabetes, aldehyde fuchsin; destruction of most B-cells, marked decrease of insulin content in B-cells; ×280; (d) intact, diethyl pseudoisocyanine; usual normal insulin content in B-cells (bright red fluorescence); ×140; (e) mild form of diabetes, diethylpseudoisocyanine; visually undetectable decrease in insulin content in B-cells; ×140; (f) heavy form of diabetes, diethylpseudoisocyanine; a fragment of a destroyed islet; marked decrease of insulin content in B-cells; ×140

to previously described changes [35], [36]. Significant changes of glycemia and LPO products were observed in experimental animals of the 1st group 60 days after administration of the "iodine-selenium" concentrate, which was confirmed by a significant decrease of blood glucose concentration, as well as the total and individual products of FRO in erythrocytes. Along with a decrease in DC, other LPO products as MDA, SPP, and SVP underwent unidirectional changes. Moreover, a decrease in the level of MDA, SPP, and SVP indicates the stabilization of cellular metabolism in rats with mild alloxan diabetes.

Analysis of the parameters of AOD enzymes in the blood of experimental animals of this group of experiments showed a more significant increase in the level of GPO in compared with SOD and catalase. Administration of the "iodine-selenium" concentrate to experimental rats with a heavy of alloxan diabetes accompanied by marked significant decrease of blood glucose level as of, the primary products of LPO (both SPP and DC), while the rate of decrease of toxic derivatives of FRO in relation to the secondary products of lipid peroxidation practically leveled off. It is characteristic that the rate of decrease in lipid peroxidation metabolites in experimental rats of Group 1 was higher in erythrocytes than in plasma, while in experimental animals of Group 2, the opposite tendency was observed. The ability of the "iodine-selenium" concentrate to inhibit LPO processes in animals with heavy alloxan diabetes is more pronounced in blood plasma, which is most likely due to both the rigidity of erythrocyte membranes, subject to destructive changes. Analysis of the dynamics of changes in AOD enzymes in experimental rats of the second group animals showed a significant increase in the level of GPO in the blood, since this enzyme belongs to a selenium-containing enzyme, however, the activity of the enzyme was lower than in animals of the first group of the experiment, which can be explained by a more severe the course of diabetes in experimental rats and disruption of the glutathione-dependent system [37], [38], [39] of antioxidant protection and a decrease in the transport properties of iodides. The obtained results showed, therefore, that prolonged administration of the "iodine-selenium" concentrate to rats with mild experimental diabetes mellitus is accompanied by a preventive effect in relation to this metabolic pathology, clearly confirmed by the results of histological analysis of pancreatic tissue and insulin and zinc content in B-cells. It was shown that in experimental animals, the histostructure of islets retained its usual structure with a slight decrease of insulin content in B-cells, which was not clearly revealed by visual examination, while the study of the histostructure of the pancreas and insulin content in B cells in experimental rats of Group 2 allowed to state.

Thus, obtained data indicate that the preventive effect of the iodine-selenium concentrate in relation to the development of a mild form of alloxan diabetes is due to the antioxidant properties of selenium, as well as, probably, its ability to potentiate the activity of GPO,

which blocks lipid peroxide and hydrogen peroxide in alloxan diabetes mellitus as and perhaps also stimulate the transport of selenium into the cell membrane of the pancreas.

Conclusions

In analogical experimental conditions in animals with a heavy course of diabetes, the development of diabetes could not be prevented despite a significant improvement in a number of indicators. To a certain extent, this was facilitated by high values of indicators (blood glucose,

KD, MDA, SPP, and SVP products in erythrocytes, insulin, and zinc content in B-cells of pancreatic islets), as well as the presence of irreversible histological changes in the islets, which made it impossible to provide a preventive effect.

The warning effect of the iodine-selenium concentrate in rats with mild alloxan diabetes is probably due to the antioxidant effect of selenium, its ability to potentiate the activity of GPO, which blocks lipid peroxides and hydrogen peroxide in alloxan diabetes and also stimulate iodine-ion transport of selenium into the cell membrane of the pancreas.

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