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Content of highly toxic metabolites in blood plasma and retina of experimental animals with streptozotocin diabetes upon exposure to lipoic acid and quercetin

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Introduction. The relevance of the work lies in studies of the effect of quercetin and lipoate in treatment of experimental diabetes.

Purpose. To examine the content of highly toxic metabolites in blood plasma and retina of experimental animals with streptozotocin diabetes upon exposure to lipoic acid and quercetin

Methods. The studies were conducted on white rats. Experimental animals were divided into four groups: first - control group (14 rats); second - experimental group (14 rats), diabetes without the use of drugs; third - experimental group (12 rats), diabetes, and the use of lipoic acid; fourth - experimental group (15 rats), diabetes, and the use of quercetin. Determination of methylglyoxal and acetoacetate has been produced in the homogenates of retinas and in blood plasma.

Results. When using lipoic acid and quercetin, a significant increase of high-reactive compounds level has been observed in the retina of experimental animals in streptozotocin diabetes.

Conclusion. It has been observed a significant increase of the level of acetoacetate and methylglyoxal in the retina of rats with streptozotocin diabetes that was particularly pronounced 6 months after the beginning of the experiment. High concentrations of methylglyoxal in the retina can be considered as an important pathochemical element of the mechanism of destruction of this visual analyzer structure in diabetes. Administration of lipoic acid and quercetin medicines when modeling streptozotocin diabetes leads to an expressed reduction of methylglyoxal, and acetoacetate concentration in the blood and the retina of experimental animals.

Introduction

Absence of effective mode of therapy and prevention of such a difficult complication of diabetes mellitus as diabetic retinitis determines the necessity to conduct detailed pathogenesis investigations of this disease [2, 4, 10, 19, 25].

A special interest in terms of release mechanisms for vascular endothelium damage by diabetes mellitus is attached to oxidative stress condition connected primarily with increased level of free radical oxygen intermediates. Initially, as a rule, an increased generation of these compounds is due to mitochondria malfunction that represent power plants of the cell. In this regard the condition of mitochondria in the retina in diabetes deserves a special attention. The role of these ultra-structures in the advanced generation of active oxygen forms in the retina in diabetes is actively studied in the investigations over the last few years [5, 11, 21, 22].

In recent years, an increasingly greater attention during the study of pathogenesis of diabetic diseases of vascular and nervous systems is attracted by release metabolic mechanisms that lead to formation of glycation end products and protein kinase activation C [14, 18].

An increased level of not only glucose, but of a wide range of metabolites of carbohydrate-phosphorus and lipid metabolism, including first of all acetoacetate, methyl glyoxal, diglycerol, sorbitol, deoxyglucose and others are considered as release metabolic disorders that favor a damage of vascular, nervous and other body tissues [20, 23, 24].

At present time, early (compounds of glucose with proteins and hydroxy aldehydes – methyl glyoxal)

and end products of nonenzymatic glycosylation of proteins, lipids, nucleic acids are considered as main pathochemical metabolites [1, 3].

Potential mechanisms of pathological influence of methyl glyoxal on the organism are various. First of all, it should be noted that methyl glyoxal is able to react with initial amino groups of various protein molecules, and it also able to selectively interact with molecules that contain guanidine arrangement, for example, with arginine [13, 16].

Significant increase of methyl glyoxal concentration can cause inactivation of glycolytic enzymes in neutrophils, the majority of which need the presence of free matters of arginine for functioning [15]. First of all, inhibition of these enzymes contributes to slowdown of the biosynthesis processes and reduction of glycogen content and, as a consequence, leads to decrease a phagocytic activity, chemotaxis and several other metabolic and functional disorders in the neutrophils in diabetes mellitus [17].

It has been shown in experimental investigations that a significant increase of acetoacetate and methyl glyoxal levels has been observed in the retina of rats having streptozotocin diabetes; it was especially expressed in 4 weeks – coenzymes given to such animals lead to a vivid decrease of acetoacetate and methyl glyoxal concentration in blood and retina [8].

In this regard, the investigations that are aimed at development of methods of decrease of formation and deactivation of these highly reactive metabolites acquire a special actuality.

Having regard to the above, we carried the study of concentration of highly reactive metabolites acetoacetate and methyl glyoxal in the blood and retina of rats during streptozotocin diabetes development.

Work objective: to study the content of high-toxic metabolites in blood plasma and retina of experimental animals with streptozotocin diabetes upon exposure to lipoic acid and quercetin.

Material and methods

The investigations were carried on albino Wistar rats weighing 190 – 210 g.

Experimental animals were divided into four groups: first – control group (14 rats), second – experimental group (14 rats), animals with developing diabetes, without drug administration, third – experimental group (12 rats), animals with developing diabetes and lipoic acid administration, fourth – experimental group (15

rats), animals with developing diabetes and quercetin administration.

Quercetin and lipoic acid were administered to the animals with developing diabetes orally during the whole experimental period (6 months).

During the performance of the experiment, the recommendations accepted by international community for visual organ study with regard to the investigations of animals were followed.

The diabetes was induced by means of streptozotocin injection (55 mg on 1 kg of body weight, intraperitoneal). Aiming to prevent weight reduction in sustention of hyperglycosemia (blood sugar level ranged from 20 till 25 mM), insulin was injected to the animals.

Upon expiration of two months of diabetes development, a part of animals (separate groups) that participated in the experiment conditions and also normal rats (control) were decapitated with the preliminary use of the anesthetic: thiopental sodium (50 mg of the drug for kg of weight). The eyes were enucleated on the ice at a temperature of 0–5°C.

Upon expiration of six months of diabetes development, the remaining part of animals was also slaughtered in accordance with the rules of work with experimental animals. The removed retina of animals was immediately exposed to studying.

The determination of acetoacetate and methyl glyoxal content in the homogenates of the retina and blood plasma has been conducted.

The determination of acetoacetate and methyl glyoxal has been done under the established procedure [7,12].

The received data underwent statistical analysis with SPSS 11.0 packet [6].

Results and their discussion

It should be noted that upon 2 months of diabetes development, the content of methyl glyoxal in the blood plasma of albino rats with streptozotocin diabetes before the drug administration was increased by 106.7% (0.155 ± 0.011) nmol/ml in relation to norm (0.075 ± 0.006) nmol/ml ($p < 0.001$), acetoacetate content – by 81.4% in relation to norm (0.097 ± 0.008) mcmol/ml ($p < 0.001$).

After the administration of lipoic acid the content of methyl glyoxal in the blood plasma of these animals decreased to (0.122 ± 0.008) nmol/ml, the decrease amounted to 21.3% in relation to diabetic animals group without drug administration (0.155 ± 0.011) nmol/ml ($p < 0.05$); after the administration of acetoacetate - to (0.135 ± 0.010) mcmol/ml that amounted to 76.7% in relation to group «without drug» (0.176 ± 0.012) mcmol/ml ($p < 0.05$) (table 1).

Experimental Studies

Table 1. Content of methyl glyoxal (nmol/ml) and acetoacetate (mcmol/ml) in the blood plasma of albino rats with streptozotocin diabetes 2 months after the influence of lipoic acid and quercetin

Biochemical values	Statistical figures	Norm n=14	Study groups		
			Without drug n=14	Lipoic acid n=12	Quercetin n=15
Methyl glyoxal	M	0.075	0.155	0.122	0.128
	m	0.006	0.011	0.008	0.007
	p ₁	-	<0.001	<0.001	<0.001
	% ₁	100,0	206.7	162.7	170.7
	p ₂	-	-	<0.05	<0.05
	% ₂	-	100.0	78.7	82.5
Acetoacetate	M	0.097	0.176	0.135	0.140
	m	0.008	0.012	0.010	0.009
	p ₁	-	<0.001	<0.01	<0.01
	% ₁	100.0	181.4	139.2	144.3
	p ₂	-	-	<0.05	<0.05
	% ₂	-	100.0	76.7	79.5

Note: p₁-level of significance of data difference in relation to the norm; p₂-level of significance of data difference in relation to the group "without drug"

Table 2. Content of methyl glyoxal (nmol/g) and acetoacetate (mcmol/g) in the retina of albino rats with streptozotocin diabetes 2 months after the influence of lipoic acid and quercetin

Biochemical values	Statistical figures	Norm n=14	Study groups		
			Without drug n=14	Lipoic acid n=12	Quercetin n=15
Methyl glyoxal	M	0.096	0.172	0.144	0.147
	m	0.007	0.013	0.010	0.011
	p ₁	-	<0.001	<0.001	<0.001
	% ₁	100.0	179.2	150.0	153.1
	p ₂	-	-	>0.05	>0.05
	% ₂	-	100.0	83.7	85.5
Acetoacetate	M	0.129	0.213	0.174	0.180
	m	0.007	0.014	0.012	0.013
	p ₁	-	<0.001	<0.01	<0.01
	% ₁	100.0	165.1	134.9	139.5
	p ₂	-	-	<0.05	>0.05
	% ₂	-	100.0	81.7	84.5

Note: p₁-level of significance of data difference in relation to the norm; p₂- level of significance of data difference in relation to the group "without drug"

After the administration of quercetin, the content of methyl glyoxal in the blood plasma decreased in relation to group of animals «without drug» by 17.5% (0.128 ± 0.007) nmol/ml ($p < 0.05$), and acetoacetate content – by 20.5%, comprising (0.140 ± 0.009) mcmol/ml ($p < 0.05$) (table 1).

The content of methyl glyoxal in the retina of albino rats with streptozotocin diabetes without drug increased by 79.2% in relation to norm (0.096 ± 0.007) nmol/g ($p < 0.001$), the content of acetoacetate – by 65.1% in relation to norm (0.129 ± 0.007) mcmol/g ($p < 0.001$).

The level of methyl glyoxal in the retina during the experimental development of diabetes and lipoic acid administration in 2 months decreased by (0.144 ± 0.010) nmol/g that made 83.7% in relation to group of diabetic animals without drug administration (0.172 ± 0.013) nmol/g, and during the quercetin administration the content of methyl glyoxal decreased by 14.5%, making (0.147 ± 0.011) nmol/g, in relation to the group of animals «without drug» (table 2).

The content of acetoacetate in the retina of albino rats with streptozotocin diabetes in 2 months

Table 3. Content of methyl glyoxal (nmol/ml) and acetoacetate (mcmol/ml) in the blood plasma of albino rats with streptozotocin diabetes 6 months after the influence of lipoic acid and quercetin

Biochemical values	Statistical figures	Norm n=14	Study groups		
			Without drug n=14	Lipoic acid n=12	Quercetin n=15
Methyl glyoxal	M	0.075	0.172	0.130	0.136
	m	0.006	0.012	0.009	0.010
	p ₁	-	<0.001	<0.001	<0.001
	% ₁	100.0	229.3	173.3	181.3
	p ₂	-	-	<0.05	<0.05
	% ₂	-	100.0	75.5	79.1
Acetoacetate	M	0.097	0.198	0.141	0.148
	m	0.008	0.014	0.010	0.012
	p ₁	-	<0.001	<0.01	<0.01
	% ₁	100.0	204.1	145.4	152.6
	p ₂	-	-	<0.01	<0.01
	% ₂	-	100.0	71.2	74.7

Note: p₁-level of significance of data difference in relation to the norm; p₂- level of significance of data difference in relation to the group "without drug"

after the lipoic acid administration decreased to (0.174±0.012) mcmol/g that amounted to 81.7% in relation to group «without drug» (0.213±0.014) mcmol/g (p<0.05), and during the quercetin administration - by 84.5% that made (0.180±0.013) mcmol/g in relation to the group of animals «without drug» (p<0.05) (table 2).

In 6 months after the diabetes development, the content of methyl glyoxal in the blood plasma of experimental animals without drug administration was increased by 129.3% in relation to the norm (0.075±0.006) nmol/ml, and the content of acetoacetate - to 104.1% in relation to the norm (0.097±0.008) mcmol/ml (p<0.001).

In the course of diabetes development, 6 months after the lipoic acid administration, the content of methyl glyoxal in the blood plasma of experimental animals decreased by 24.5% – to (0.130±0.009) nmol/ml in relation to group «without drug» (0.172±0.012) nmol/ml (p<0.05), and during the administration of quercetin the content of methyl glyoxal decreased by 20.9% to (0.136±0.010) nmol/ml in relation to group of animals without drug (p<0.05) (table 3).

The level of acetoacetate in the blood plasma of experimental animals during diabetes development in 6 months after lipoic acid administration decreased by 71.2% to (0.141±0.010) mcmol/ml in relation to group of diabetic animals without drug administration (0.198±0.014) mcmol/ml (p<0.01). Quercetin administration led to decrease of acetoacetate level in blood plasma of the

experimental animals by 25.3% – to (0.148±0.012) mcmol/ml in relation to group «without drug» (table 3).

The content of methyl glyoxal in the retina of albino rats with streptozotocin diabetes without drug in 6 months was increased by 110.4%, making (0.202±0.014) nmol/g in relation to the norm (0.096±0.007) nmol/g (p<0.001), and the content of acetoacetate – by 89.9%, making (0.245±0.015) mcmol/g in relation to norm (0.129±0.007) mcmol/g (p<0.001).

Under conditions of lipoic acid administration in six months after diabetes development the content of methyl glyoxal in the retina of experimental animals decreased to (0.157±0.010) nmol/g, i. e. by 22.3% in relation to group «without drug» (p<0.05), and during the quercetin administration - to (0.164±0.011) nmol/g that made 18.8% in relation to the group of animals that did not receive the drug (p<0.05) (table 4).

The content of acetoacetate in the retina of experimental animals during the diabetes development in 6 months after the lipoic acid administration decreased to (0.186±0.012) mcmol/g that made 75.9% in relation to the group «without drug» (0.245±0.015) mcmol/g (p<0.01), and during the quercetin administration - by 21.2% that made (0.193±0.014) mcmol/g (p<0.05) in relation to the group of animals that did not receive the drug (p<0.05) (table 4).

In such a way the content of methyl glyoxal and acetoacetate in the blood plasma and retina of

Table 4. Content of methyl glyoxal (mcmol/g of tissue) and acetoacetate (mcmol/g of tissue) in the retina of albino rats with streptozotocin diabetes 6 months after the influence of lipoic acid and quercetin

Biochemical values	Statistical figures	Norm n=14	Study groups		
			Without drug n=14	Lipoic acid n=12	Quercetin n=15
Methyl glyoxal	M	0.096	0.202	0.157	0.164
	m	0.007	0.014	0.010	0.011
	p ₁	-	<0.001	<0.001	<0.001
	% ₁	100.0	210.4	163.5	170.8
	p ₂	-	-	<0.05	<0.05
	% ₂	-	100.0	77.7	81.2
Acetoacetate	M	0.129	0.245	0.186	0.193
	m	0.007	0.015	0.012	0.014
	p ₁	-	<0.001	<0.001	<0.001
	% ₁	100.0	189.9	144.2	149.6
	p ₂	-	-	<0.01	<0.05
	% ₂	-	100.0	75.9	78.8

Note: p₁-level of significance of data difference in relation to the norm; p₂- level of significance of data difference in relation to the group "without drug"

experimental animals with diabetes without drugs administration in 2 months was over the limit and increased over time of monitoring (in 6 months). The administration of lipoic acid and quercetin to the experimental animals with diabetes led to the decrease of the content of methyl glyoxal and acetoacetate in the blood plasma and retina.

The increase of the level of highly reactive compounds in the retina can cause damage of vascular endothelium and directly of retina tissues biostructures, leading to accumulation of glycation end products that can be considered as one of leading release mechanisms of diabetic retinitis development. The data of series of studies showed that the metabolites we examine can directly influence on protein and membrane structures of retina neurepithelium. The results we obtained are consistent to a considerable degree with the data of experimental investigations of oxoaldehydes study in patients with diabetic retinitis obtained by Pavlyuchenko CP, Oleynik TV, Mogilevskiy SYu and Chuyko AL [8,9].

The increase of highly reactive metabolites: methyl glyoxal and acetoacetate in the blood and ocular tissues when modeling streptozotocin diabetes can be considered as a fundamental element in the mechanism of development of different metabolic disorders, systems of humoral

and cell-bound immunities, structural functional status of the blood stream and the nervous system tissues. Methyl glyoxal, acetoacetate and other highly reactive compounds of the carbohydrate-phosphorus metabolism cause chemical modification of proteins – structural and regulatory ones. It is the damage of protein structures that is not-reversible can be considered as a leading initiating mechanism in the process of development of different complications in diabetes mellitus, in general, and in diabetic retinitis development, in particular.

Conclusions

1. A significant level increase of methyl glyoxal and acetoacetate, especially expressed in 6 months after the start of the experiment, has been noted in the retina of rats with streptozotocin diabetes. High concentrations of methyl glyoxal in the retina can be considered as an important pathochemical element of the damage mechanism of this visual analyzer structure in diabetes.

2. The administration of drugs of lipoic acid and quercetin when modelling the streptozotocin diabetes leadstoexpressed decrease of concentration of methyl glyoxal and acetoacetate in the blood and the retina of experimental animals.

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