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EXPERIMENTAL TYPE 2 DIABETES MELLITUS AND ACETAMINOPHEN TOXIC LESIONS: GLUTATHIONE SYSTEM INDICES CHANGES

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Abstract

Background. The goal of the research was to study the effect of acetaminophen on major glutathione part of antioxidant system indices in liver homogenate of rats with type 2 diabetes mellitus in time dynamics. **Materials and methods**. We conducted two series of experiments. In the first series toxic lesion was caused by a single intragastric administration of acetaminophen suspension in 2 % starch solution to animals in a dose of 1250 mg/kg (1/2 LD_{50}). In the second series the suspension of acetaminophen in 2 % starch solution in a dose of 55 mg/kg was given, which corresponds to the highest therapeutic dose during 7 days. Non-genetic form of experimental type 2 diabetes mellitus was modeled by Islam S., Choi H. method (2007). Activity of glutathione peroxidase (GPx) and glutathione reductase (GR), and contents of reduced glutathione (GSH) were determined in liver homogenate. **Results.** The obtained results have shown that GR and GPx activity actively decreased after acetaminophen administration in higher therapeutic doses to rats with type 2 DM. However, the changes were less pronounced than in rats with type 2 DM and acute acetaminophen toxic lesions. **Conclusion.** Results of the research have shown that acetaminophen administration to rats with type 2 DM causes a significant violation of compensatory mechanisms, especially of the enzyme and nonenzyme parts of antioxidant system.

Key words: **acetaminophen, reduced glutathione, glutathione peroxidase, glutathione reductase, diabetes mellitus.**

INTRODUCTION

The most important function of the liver in the body is neutralization and decomposition of toxic substances. Metabolism and disposal of chemical and biological toxins is carried out by decontaminating system of hepatocytes, followed by the removal of harmful products from the body [2, 3]. However, some substances, including drug substances become even more hepatotoxic than most pharmaceuticals as a result of these changes (the so-called "lethal synthesis"). Therefore, prolonged taking of such medicines leads to the depletion of neutralizing system of liver as well as hepatocytes damage [1].

Diabetes mellitus (DM) is a chronic endocrine disease [4]. The problem of diabetes mellitus is very acute not only in our society, but also in the whole world. Each 9th person suffers from this disease, of which 15-20% are children.

Nowadays there are many types of medicines that contain acetaminophen and are used mainly as antipyretics. People with diabetes mellitus often take these drugs [5, 6, 11, 12].

All things considered, the goal of our research was to study the state of glutathione part of antioxidant system in rats with type 2 diabetes mellitus and acetaminophen toxic lesions.

MATERIAL AND METHODS

The experiments were carried out on white rats weighing 180-220 g, which were on a standard diet and access to water in vivarium.

We conducted two series of experiments. In the first series toxic lesion was caused by a single intragastric administration of acetaminophen suspension in 2 % starch solution to animals in a dose of 1250 mg/kg (1/2 LD_{50}). In the second series the suspension of acetaminophen in 2 % starch solution in a dose of 55 mg/kg was given, which corresponds to the highest therapeutic dose during 7 days. Non-genetic form of experimental type 2 diabetes mellitus was modeled by Islam S., Choi H. method (2007), that is, a single intraperitoneal administration of streptozotocin solution in doses 65 mg/kg («Sigma», USA) to rats weighing (200 ± 20 g), which was diluted by citrate buffer (pH 4.5) with the previous (15 minutes ahead) intraperitoneal nicotinamide administration in doses of 230 mg/kg [13]. Rats with the same body weight, which were given the same amount of solvent (citrate buffer pH 4.5), were used as the control group.

In the first series of experiments, the rats were divided into 4 groups: 1st group was intact (control); 2nd group was with a single acetaminophen administration, 3rd group were animals with type 2 diabetes mellitus caused by streptozotocin administration, and 4th group were rats with a single administration of acetaminophen after streptozotocin administration. In the second series of experiments, the rats were divided into 4 groups: 1st group was intact (control); 2nd group was with acetaminophen administration during 7 days, 3rd group were animals with type 2 diabetes mellitus caused by streptozotocin administration, 4th group were rats with administration of acetaminophen during 7 days after streptozotocin administration.

The animals were removed from the experiment on the first, third, fifth and seventh days after last acetaminophen administration by means of euthanasia under thiopental anesthesia. All experiments on rats were carried out according to the "Scientific and practical recommendations as how to keep laboratory animals and work with them" [7].

Activity of glutathione peroxidase (GPx) and glutathione reductase (GR), and contents of reduced glutathione (GSH) were determined in liver homogenate [8,10]. Quantitative indices were processed statistically. Results of experiments were processed by means of statistical program Statistica using parametric Student's t test and Wilcoxon signed-rank test for non-parametric statistical hypothesis test [9]. Changes were considered significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Activation of lipid peroxidation reactions is one of the fundamental biological mechanisms of biostructures damage and cellular pathology development under the influence of harmful factors of various origins, especially xenobiotics. Erythrocyte hemolysis is also an example of cell membrane damage due to formation of lipid peroxidation products, and occurs as a result of the generator NADPH deficiency, which is necessary for the glutathione reductase functioning and maintaining glutathione in the reduced form.

Glutathione is a central component of the antioxidant systems of almost all cells and organs. Its antioxidant action is associated with the transfer of sulfhydryl groups. Glutathione is converted into disulfide under glutathione peroxidase action and, as a result, lipid peroxidation products are deactivated. Glutathione reductase converts glutathione into reduced form. The activity of glutathione peroxidase in the body largely determines the dynamics of pathological processes. If the activity of glutathione peroxidase decreases, protection of liver cells from dangerous chemicals is violated.

According to the obtained results, it can be argued that in the first group of the experimental series glutathione system is exhausted in animals with lesions caused by acetaminophen and type 2 diabetes mellitus (table 1).

Table 1. Dynamics of reduced glutathione content (mg/kg), activity of glutathione reductase (mmol/(min×kg)) and glutathione peroxidase (mmol/(min×kg)) in liver homogenate of rats with type 2 diabetes mellitus and acute acetaminophen toxic lesions (M±m; n=10)

Group of animals	Time after acetaminophen administration (days)			
	1st day	3rd day	5th day	7th day
Control n = 10	GSH 3.81±0.19 GR 72.42±2.99 GPx 0.279±0.128			
Acetaminophen (single) n = 10	GSH 2.26±0.13*	GSH 2.50±0.46*	GSH 2.66±0.51	GSH 2.76±0.40*
	GR 30.41±1.15*	GR 33.53±1.64*	GR 35.56±1.29*	GR 36.73±1.99*
	GPx 0.095±0.003	GPx 0.108±0.026	GPx 0.125±0.017	GPx 0.136±0.011
Type 2 DM n = 10	GSH 3.33±0.25	GSH 3.48±0.58	GSH 3.72±0.70	GSH 3.93±0.61
	GR 71.55±3.74	GR 74.15±3.22	GR 83.49±3.73*	GR 87.33±3.28*
	GPx 0.208±0.046	GPx 0.221±0.034	GPx 0.236±0.037	GPx 0.250±0.045
Acetaminophen (rats with Type 2 DM) n = 10	GSH 1.25±0.20*	GSH 1.38±0.30*	GSH 1.48±0.30*	GSH 1.71±0.29*
	GR 27.59±1.45*	GR 31.50±1.53*	GR 34.47±1.56*	GR 36.35±2.02*
	GPx 0.080±0.021	GPx 0.093±0.021	GPx 0.109±0.021	GPx 0.122±0.023

Notes: * - significant difference if to compare with control animals;

There was a maximum decrease in reduced glutathione on the 1st day of the experiment in all groups of experimental animals: in animals with acute acetaminophen toxic lesion it decreased by 40.7 % (2nd group), in animals with type 2 DM it went down by 12.7 % (3rd group). Administration of 1/2 LD_{50} acetaminophen for rats with type 2 DM (4th group) caused even greater decrease of the index by 67.3 % in comparison with a control group of animals. Thus, these results show that greater toxicity is caused by acetaminophen administration to rats with type 2 DM and leads to dramatic decrease of GSH. On the following days of the experiment, this index was gradually reduced. On the 7th day of the experiment it was already 72.5 % in the 2nd group of animals, 44.7 % in the 4th group of animals, and in the 3rd group this index became normal. It is noteworthy that GSH content is reduced faster in animals with type 2 DM and acute acetaminophen toxic lesions, which is probably associated with greater activation of defensive processes.

The activity of GR and GPx in liver homogenate was on lower level during the whole experiment in the 2nd and 4th groups of animals. The maximum decrease of these indices, as well as of GSH content was observed on the first day of the experiment (GR activity was 42.0 % and 38.1 % respectively, GPx activity - 34.1 % and 28.7 % respectively). According to the obtained results, GPx has undergone greater inactivation than GR. Glutathione reductase activity in animals of the 3rd group decreased by 1.2 % on the 1st day, and there was an increase of this index by 2.4 %, 15.3 % and 20.6 % on the 3rd, 5th and 7th day respectively, while glutathione peroxidase activity in this group of animals decreased by 25.4 % on 1st day of the experiment, and by 20.8 % on the 3rd, by 15.4 % on the 5th and by 10.4 % on the 7th day, which means gradual recovery of enzyme activity.

As in the first series of experiments, in the second series (animals with type 2 DM and acetaminophen administration in maximum therapeutic dose during 7 days) we noticed that during 7 days glutathione peroxidase and glutathione reductase activity as well as reduced glutathione content (Table 2) decreased compared to control animals indices during the entire experiment.

Table 2. Dynamics of reduced glutathione content (mg/kg), activity of glutathione reductase (mmol/(min×kg)) and glutathione peroxidase (mmol/(min×kg)) in liver homogenate of rats with type 2 diabetes mellitus and acetaminophen administration in doses 55 mg/kg during 7 days (M±m; n=10)

Group of animals	Time after acetaminophen administration (days)			
	1st day	3rd day	5th day	7th day
Control n = 10	GSH 3.81±0.19 GR 72.42±2.99 GPx 0.279±0.128			
Acetaminophen (7 days) n = 10	GSH 3.31±0.19	GSH 3.53±0.45	GSH 3.73±0.61	GSH 3.98±0.53
	GR 48.73±1.60*	GR 51.27±1.29*	GR 53,30±1.87*	GR 57.57±2.42*
	GPx 0.142±0.009	GPx 0.153±0.016	GPx 0.171±0.023	GPx 0.182±0.020
Type 2 DM n = 10	GSH 3.33±0.25	GSH 3.48±0.58	GSH 3.72±0.70	GSH 3.93±0.61
	GR 71.55±3.74	GR 74.15±3.22	GR 83.49±3.73*	GR 87.33±3.28*
	GPx 0.208±0.046	GPx 0.221±0.034	GPx 0.236±0.037	GPx 0.250±0.045
Acetaminophen (rats with Type 2 DM) n = 10	GSH 3.01±0.34	GSH 3.11±0.40	GSH 3.27±0.52	GSH 3.40±0.62
	GR 46.78±2.23*	GR 48.23±2.07*	GR 51.34±1.85*	GR 52.94±2.38*
	GPx 0.109±0.026	GPx 0.126±0.026	GPx 0.137±0.023	GPx 0.153±0.039

Notes: * - significant difference if to compare with control animals;

As shown in the Table 2, GSH content in each experimental group was decreasing during all days of the experiment, with the exception of the 2nd and 3rd groups on the 7th day, where it increased by 3.1 % and 4.4 % respectively.

The obtained results have shown that GR and GPx activity actively decreased after acetaminophen administration in higher therapeutic doses to rats with type 2 DM. However, the changes were less pronounced than in rats with type 2 DM and acute acetaminophen toxic lesions.

CONCLUSION

Results of the research have shown that acetaminophen administration to rats with type 2 DM causes a significant violation of compensatory mechanisms, especially of the enzyme and nonenzyme parts of antioxidant system.

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