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GLUTATHIONE-RELATED ENZYME ACTIVITY IN RAT'S TESTES AND EPIDIDYMIS AT AN ACUTE INTOXICATION WITH A SYNTHETIC PYRETHROID DELTAMETHRIN

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ABSTRACT

The study was aimed for evaluation of glutathione-related enzyme activity in rats' testes and epididymis at an acute intoxication with a deltamethrin. The study was carried out on 24 male Wistar strain rats of body weight 240±10g arranged into 2 groups – control and test ones each of 12 specimens. The test group was exposed to an acute deltamethrin intoxication in a dose of 43.5 mg/kg per body wt. The acute deltamethrin intoxication is accompanied by the accumulation of uric acid and malondialdehyde associated with the lowering of glutathione level and glutathione peroxidase, glutathione reductase, glutathione S-transferase and gamma-glutamyl transferase activation in the testes and epididymis of rats. Activation of glutathione-related enzymes in the reproductive organs of rats exposed to an acute deltamethrin intoxication was due to the activation of free radical processes caused by the intensification of purine catabolism.

KEYWORDS: Pesticides, deltamethrin, glutathione, testes, epididymis

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INTRODUCTION

Pesticides are widely used in agriculture and for domestic needs. Deltamethrin (DM) ($C_{22}H_{19}Br_2NO_3$) is one of the most common insecticides exploiting at the present time. According to the data published DM is extremely dangerous for insects and various types of animals [1], including the mammals [2, 3] and the human beings as some scientists proved [4, 5]. The DM negative exposure on the reproductive system of the experimental animals has been noted at the papers [2, 6, 7]; this effect was expressed by the reduction of sex hormones in the blood of rats after DM injection. Histologically proved data of the testes examinations exposed to DM intoxication have indicated on DM toxicity effect on the reproductive system in rats [8]. Furthermore, it has been revealed in vitro experiments [9] that sperm incubation in the medium with different DM concentration led to a significant decrease of sperm mobility and its morphological abnormalities. The above mentioned data indicate on DM toxic effect on the reproductive system. However, there are not enough data published regarding the molecular mechanisms causingthe pathological changes in the reproductive system under DM effect. Some authors have supposed that DM toxic effect is associated with oxidative stress arising in the reproductive organs after DM administration to the experimental animals [7, 8]. However, the authors have not indicated the sources of free radicals and have not described a status of the antioxidant system, notably, the status of the glutathione antioxidant system. Therefore, it is a burning issue to study the enzymatic antioxidant system in the reproductive organs upon DM administration. The aim of our study is to assess the glutathione-related enzyme activity in the testes and epididymis in acute deltamethrin intoxication.

MATERIALS AND METHODS

The study has been carried out on 24 male Wistar rats (240±10g) at the Department of Biochemistry of the Omsk State Medical University in 2013–2014. Rats were divided into two groups: group 1 (n=12) was control and group 2 (n=12) was exposed to acute deltamethrin intoxication. Group 2 rats was orally administered DM in a dose of 43.5 mg/kg body wt (½ LD 50) via a metallic probe and group 1 rat was injected with an equivalent volume of saline. DM under the trade name ”Butox 50” manufactured by “Intervet International BV” was used during the study. Testes and epididymis were extracted 24 hours after DM administration followed by homogenization. The organs were homogenized in 0.15 M potassium chloride (KCl) at 0–2^{o}C. The suspensions were centrifuged (3000 g, 20 min) in a C–80 centrifuge (“Hospitex”). Total protein was measured in the supernatant by the biuret method, MDA was evaluated by TBA reaction, and glutathione was evaluated by reaction with 5.5-dithiobis-(2-nitrobenzoic) acid [10]. Uric acid was measured by spectrophotometry at 290 nm. Glutathione peroxidase (EC 1.11.1.9), glutathione S-transferase (EC 2.3.2.2) and glutathione reductase (EC 1.6.4.2) were evaluated by the method prescribed by Vlasova et al., 1990 [11]. “Randox” reagents were used. Optical densities were measured on an 2802S spectrophotometer (“UNICO”) and “Screen Master” biochemical analyzer (“Hospitex”). Activity of gamma-glutamyl transferase (EC 2.3.2.2) was determined applying “Chronolab” reagent kit. The experiment was conducted in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Council of Europe No123, Strasbourg, 1985). Statistics data processing was carried out using non-parametric *U*–Mann–Whitney test. Results were presented as *Me* – the median, Q_1 – the lower quartile, Q_3 – the upper quartile. The level of significance was set at $P<0.05$.

RESULTS AND DISCUSSIONS

Acute intoxication caused by DM within 24 hours induced the accumulation of uric acid in

the reproductive organs. Concentration of uric acid in the testes and epididymis of the group 2 rats was increased by 34 and 22% respectively compared with the control group (Table). It was likely associated with acute disorders of purine metabolism, the process being previously described in various pathological conditions [12, 13]. Acute disorders of purine metabolism is characterized by the damage of nucleic acids, energy metabolism disturbance followed by AMP accumulation triggering a process of profound catabolism of purine mononucleotides to uric acid. Uric acid accumulation is a marker for acute disorders of purine metabolism. Our data are matched with the studies conducted by M.M. Hossain and J.R. Richardson [14] at the cell cultures, where it has been found that DM causes DNA damages and fragmentation. One of the negative effects of enhanced purine catabolism is the activation of xanthine oxidase. The enzyme actively produces reactive oxygen species (ROS) which are able to damage lipids, proteins and nucleic acids. Excessive ROS attack on lipid membrane structures induces MDA accumulation. In our experiment, we have observed MDA increase in the testes and epididymis of rats subjected to the acute DM intoxication by 47 and 60% respectively in comparison with the control group.

Table 1
Biochemical parameters of the testes and epididymis of rats with acute intoxication deltamethrin, Me (Q_1–Q_3), n=12

Parameter	Testes		Epididymis	
	Control	AIDM	Control	AIDM
Uric acid, *nmol/mg protein*	148 (108–163)	198 (168–215) pU=0.0019	122 (102–143)	149 (140–170) pU=0.0043
MDA, *nmol/mg protein*	50.1 (37.8–65.8)	73.6 (65.0–88.7) pU=0.0052	42.6 (31.5–54.8)	67.9 (51.7–78.4) pU=0.0045
Glutathione, *nmol/mg protein*	18.6 (14.1–23.8)	11.9 (10.1–13.3) pU=0.0046	16.5 (11.5–18.2)	10.6 (8.78–13.5) pU=0.0082
Glutathione peroxidase, *U/mg protein*	511 (410–622)	680 (598–933) pU=0.0078	299 (272–357)	379 (361–426) pU=0.0023
Glutathione reductase, *U/mg protein*	314 (266–352)	489 (379–601) pU=0.0093	241 (209–272)	356 (316–480) pU=0.0012
Glutathione S-transferase, *U/mg protein*	296 (219–413)	401 (363–536) pU=0.0192	695 (528–885)	963 (882–1249) pU=0.0037
Gamma-glutamyl transferase, *U/mg protein*	402 (318–507)	947 (778–1278) pU=0.0014	220 (187–236)	338 (295–447) pU=0.0016

Note. AIDM – acute deltamethrin intoxication; pU – statistically significant differences vs control

Accumulation of uric acid and MDA in the reproductive organs of rats exposed to acute DM intoxication testifies the significant activation of free radical processes. In this case, glutathione is one of the principal metabolites fighting against ROS excess. Its rate in the testes and epididymis of group 2 rats is reduced by 36 and 35% respectively in comparison with the same index in group 1 rats (Table). Glutathione peroxidase, glutathione reductase, gamma-glutamyl transferase and other enzymes regulate glutathione level in the cell. Glutathione peroxidase, glutathione and different antioxidant enzymes effectively combat against ROS excess. It has been observed the increasing of glutathione

peroxidase activity in the testes and epididymis in group 2 rats by 33 and 27% respectively compared with the control group (Table1). To our opinion, it is a cell response to the increasing of ROS rate. Glutathione peroxidase activation leads to the reducing of glutathione level in the reproductive organs in acute DM toxicity as noted above. In response to oxidized cellular glutathione (GSSG) accumulation, the increasing of glutathione reductase activity occurs. The activity of this enzyme in the testes and epididymis in the group 2 rats is by 56 and 48% higher than in the group 1 respectively. Glutathione reductase functioning depends on reduced NADPH in the cell. Prolonged glutathione reductase activation may provoke the imbalance in NADPH–$NADP^+$ ratio in the cell and negatively effect on the inactivation of DM and other toxic substances entering into the body of humans and animals every day. Glutathione S-transferase is one of the glutathione metabolism enzyme involved in the conversion of many xenobiotics, including deltamethrin [15]. The activity of this enzyme in the testis and epididymis of rats in Group 2 is higher at 36 and 39% respectively than in the control, indicating an intensive involvement of glutathione S-transferase in the process of inactivation of both the deltamethrin and lipid peroxidation products that were produced as a result of an acute purine metabolism disorder. Glutathione S-conjugates which were produced in a reaction catalyzed by glutathione S-transferase tend to further alteration upon action of a number of enzymes with gamma-glutamyl transferase as well. In our study there was an increase in the activity of this enzyme in the testis and epididymis of rats in group 2 at 136 and 134% respectively, compared to the same period in the control (Table 1.). Intensive use of glutathione in reactions involved glutathione S-transferase and gamma-glutamyl transferase promotes its deficiency. For glutathione resynthesis it requires energy of ATP, the availability of which may be limited due to increased catabolism of purine mononucleotides during acute disorders of purine metabolism.

CONCLUSION

Hence, single administration of deltamethrin in a dose of 43.5 mg/kg body wt caused the accumulation of uric acid and malondialdehyde in the testes and epididymis of rats associated with the decreasing of glutathione level. Acute deltamethrin intoxication is accompanied by glutathione peroxidase, glutathione reductase and gamma-glutamyl transferase activation in the testes and epididymis of rats. Glutathione-related enzyme metabolism in the reproductive organs of rats exposed to acute deltamethrin intoxication is associated with the activation of free radical processes caused by purine catabolism enhancement.

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