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Trigger Mechanisms of Cypermethrin-Induced Changes of Metabolism: An Experimental Study

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Abstract

Background: The purpose of this work was to study the triggering mechanisms of metabolic changes in experimental animals after a single injection of cypermethrin at a dose of 55 mg/kg of body weight.

Methods and Results: Sixty rats were randomly divided into four groups (15 rats in each group). Rats of the control groups (G1 and G3) were injected with saline into the stomach. Animals of the experimental groups (G2 and G4) were injected once with the synthetic pyrethroid cypermethrin at a dose of 55 mg/kg of body weight, which is 1/5 LD50.

Before being withdrawn from the experiment, blood was taken in vivo under anesthesia, and the liver was removed. The glucose, lactate, uric acid, and total bilirubin concentrations were determined in blood serum by unified research methods. The content of glutathione (GSH), malondialdehyde (MDA), and activity of glucose-6-phosphate dehydrogenase (G6PDH) was determined in erythrocyte hemolysates. In liver homogenates, the content of total protein, glycogen, uric acid, and inorganic phosphorus (Pi) was determined by unified methods, as well as MDA, GSH, activity of G6PDH, microsomal oxygenase, glutathione-S-transferase (GST), glutathione peroxidase (GPx), and glutathione reductase (GR).

Administration of cypermethrin to laboratory rats at a dose of 1/5 LD50 causes adaptive changes in metabolism. After one day, there was an increase in the content of glucose in the blood serum against the background of a deficiency of carbohydrates in the liver tissue. At the same time, there was an increase in anaerobic oxidation and an increase in purine catabolism, which was associated with the activation of lipid peroxidation of cell membranes and the depletion of the pool of antioxidants. GSH deficiency was exacerbated by an increase in the activity of antioxidant enzymes and xenobiotic biotransformation systems. Seven days after the administration of cypermethrin, rats retained a high rate of breakdown of purines to uric acid. This process was enhanced by a decrease in the red blood cells, a deficiency of carbohydrates, and inhibition of the activity of G6PDH, GPx, and GR. This ultimately led to the development of oxidative stress.

Conclusion: The triggers for the development of oxidative stress under cypermethrin exposure are lactic acidosis and increased catabolism of purine mononucleotides, accompanied by an increase in the production of free radicals and inhibition of the function of the antioxidant system. A decrease in the blood red blood cells, carbohydrate deficiency, and suppression of the activity of the pentose cycle 7 days after the administration of cypermethrin aggravate this condition.(**International Journal of Biomedicine. 2023;13(2):309-312.**)

Keywords: purine metabolism • carbohydrate metabolism • oxidative stress • cypermethrin • liver • rats

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Abbreviations

ATP, adenosine triphosphate; **G6PDH**, glucose-6-phosphate dehydrogenase; **GPx**, glutathione peroxidase; **GR**, glutathione reductase; **GSH**, glutathione; **GST**, glutathione-S-transferase; **LP**, lipid peroxidation; **MDA**, malondialdehyde; **NADPH**, nicotinamide adenine dinucleotide phosphate; **Pi**, inorganic phosphate; **RBC**, red blood cells; **ROS**, reactive oxygen species.

Introduction

Synthetic pyrethroid cypermethrin is widely used in medicine, veterinary medicine, agriculture, and everyday life.[1-3] A large number of preparations with insecticidal and acaricidal properties have been developed on this basis.[4] In recent years, a number of scientific studies have been published, noting the low selectivity and toxicity of cypermethrin for humans and warm-blooded animals.[5,6] Many authors emphasize that under the action of synthetic pyrethroids on the mammal's body, oxidative stress has been developed.[7-9] Still, they do not cover the issue of triggering mechanisms for the development of metabolic disorders.

The purpose of this work was to study the triggering mechanisms of metabolic changes in experimental animals after a single injection of cypermethrin at a dose of 55 mg/kg of body weight.

Materials and Methods

The experiment was performed on Wistar male rats weighing 230-250 g. All stages of the experiment were carried out in accordance with the requirements of Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

Sixty rats were randomly divided into four groups (15 rats in each group). Rats of the control groups (G1 and G3) were injected with saline into the stomach. Animals of the experimental groups (G2 and G4) were injected once with the synthetic pyrethroid cypermethrin at a dose of 55 mg/kg of body weight, which is 1/5 LD50. Animals were withdrawn from the experiment in two stages: rats of G1 and G2 - in a day and rats of G3 and G4 - seven days after the start of the experiment. During the experiment, we used a preparative form of cypermethrin with the trade name "Sharpei" (CJSC "August", Russia).

Before being withdrawn from the experiment, blood was taken in vivo under anesthesia, and the liver was removed. Then the blood was centrifuged, hemolysates were prepared from the erythrocyte mass, and homogenates were prepared from the liver at 0-2°C. Biochemical parameters were determined in the obtained blood serum, erythrocyte hemolysates, and liver homogenates. The number of RBCs was determined in whole blood. The glucose, lactate, uric acid, and total bilirubin concentrations were determined in blood serum by unified research methods. The content of glutathione (GSH),[10] malondialdehyde (MDA),[11] and activity of glucose-6-phosphate dehydrogenase (G6PDH)[12] was determined in erythrocyte hemolysates. In liver homogenates, the content of total protein, glycogen, uric acid, and inorganic phosphorus (Pi) was determined by unified methods, as well as MDA,[11] GSH,[10] activity of G6PDH,[12] microsomal oxygenase, glutathione-S-transferase (GST),[13] glutathione peroxidase (GPx), and glutathione reductase (GR).[14]

Statistical analysis was performed using the STATISTICA 10 software package (Stat-Soft Inc., USA). For descriptive analysis, results are presented as median (Me), first quartile (Q1) and third quartile (Q3). Differences of continuous variables were tested by the Mann-Whitney *U*-test. A probability value of $P<0.05$ was considered statistically significant.

Results

The administration of cypermethrin to rats at a dose of 1/5 LD50 caused, after one day, an increase in levels of glucose, lactate, and uric acid in the blood serum (Table 1). In erythrocytes, there was a decrease in the concentration of GSH and accumulation of MDA. The activity of erythrocyte G6PDH remained at the level of control values (Table 1). The amount of RBC in the blood one day after the administration of cypermethrin did not change statistically significantly.

Table 1.

Changes in blood parameters in rats after a single injection of cypermethrin at a dose of 1/5 LD50.

Parameters	One day after		Seven days after	
	Group 1	Group 2	Group 3	Group 4
Blood				
RBC, $\times10^{12}$/L	8.94 (8.53; 9.33)	8.66 (8.23; 8.90) P=0.120	8.90 (8.43; 9.62)	7.82 (7.20; 8.10) P<0.001
Serum				
Glucose, mg/dL	121 (108; 134)	147 (129; 160) P=0.010	119 (103; 137)	94.3 (76.1; 115) P=0.003
Lactate, mmol/L	6.27 (5.88; 7.60)	7.91 (7.11; 8.60) P=0.002	6.20 (5.25; 7.11)	7.64 (7.23; 8.82) P<0.001
Uric acid, µmol/L	71.8 (65.2; 85.3)	93.2 (83.6; 99.2) P=0.001	78.2 (65.6; 91.2)	94.5 (88.5; 116) P=0.003
Total bilirubin, µmol/L	2.73 (2.43; 3.34)	2.82 (2.45; 3.58) P=0.672	2.56 (2.21; 2.83)	3.72 (3.21; 3.92) P=0.001
Erythrocytes				
GSH, nmol/mg protein	9.27 (8.38; 10.3)	6.35 (6.01; 7.18) P<0.001	9.30 (8.01; 11.0)	5.99 (4.95; 7.05) P<0.001
MDA, nmol/mg protein	3.09 (1.87; 3.96)	4.49 (3.62; 5.96) P=0.008	2.66 (1.69; 4.00)	5.26 (4.21; 5.99) P<0.001
G6PDH, U/mg protein	8.40 (7.92; 11.9)	8.11 (7.28; 10.7) P=0.326	10.2 (8.61; 11.9)	7.83 (5.43; 8.61) P=0.003

In the liver of the G2 rats, we found a decrease in the content of glycogen and GSH against the background of an increase in the concentration of uric acid, Pi, and MDA (Table 2), which indicated an increase in the catabolism of purine mononucleotides and an increase in lipid peroxidation (LP) of the membrane structures of hepatocytes. The activity of

enzymes of the glutathione system (GST, GPx, and GR) in rats of the G2 increased, indicating activation of the detoxifying and antioxidant functions of the liver; however, the activity of liver G6PDH did not differ from the values of this indicator in the G1 (Table 2).

Table 2.

Biochemical parameters of the liver of rats after a single injection of cypermethrin at a dose of 1/5 LD50.

Parameters	One day after		Seven days after	
	Group 1	Group 2	Group 3	Group 4
Glycogen, mg/g wet wt	51.6 (35.8; 60.8)	32.3 (23.5; 36.3) *P*=0.007	49.3 (38.1; 58.9)	31.2 (28.1; 37.8) *P*=0.002
Uric acid, µmol/g wet wt	23.8 (18.6; 30.4)	37.1 (32.1; 42.4) *P*=0.001	18.7 (16.0; 25.5)	44.2 (41.1; 53.1) *P*<0.001
Pi, µmol/g wet wt	11.4 (8.94; 15.0)	17.0 (15.2; 19.9) *P*=0.002	9.14 (7.59; 13.2)	20.6 (18.6; 26.1) *P*<0.001
MDA, nmol/ mg protein	89.5 (64.9; 91.9)	108 (95.1; 121) *P*=0.003	74,0 (65.5; 98.1)	128 (114; 151) *P*<0.001
GSH, nmol/ mg protein	38.5 (31.2; 40.3)	23,3 (20.1; 27.9) *P*<0.001	33,8 (31.0; 37.6)	21.6 (18.8; 27.4) *P*<0.001
G6PDH, U/mg protein	26.8 (24.9; 35.9)	23.3 (20.2; 32.0) *P*=0.149	31.4 (25.9; 37.0)	17.2 (13.0; 20.9) *P*=0.001
MO*, nmol/mg/min	0.570 (0.510;0.615)	0.764 (0.710;0.852) *P*<0.001	0.574 (0.504;0.628)	0.685 (0.622;0.784) *P*=0.005
GST, U/mg protein	614 (504; 680)	863 (723; 945) *P*<0.001	588 (512; 706)	778 (699; 898) *P*=0.001
GPx, U/mg protein	814 (644; 853)	1162 (948; 1372) *P*<0.001	685 (620; 846)	515 (381; 731) *P*=0.024
GR, U/mg protein	438 (368; 531)	640 (530; 828) *P*<0.001	399 (357; 444)	274 (171; 331) *P*=0.001

**MO - microsomal oxygenase*

In the blood, 7 days after the cypermethrin administration, we found a decrease in the number of erythrocytes and the development of glucose deficiency (Table 1). The blood concentration of lactic and uric acids, as well as the content of total bilirubin, on the contrary, statistically significantly exceeded the control values. GSH deficiency and decreased G6PDH enzymatic activity developed in erythrocytes against the background of MDA accumulation (Table 1).

In the liver of the G4 rats, a statistically significant decrease in the concentration of glycogen and GSH was detected (Table 2), while the content of uric acid, Pi, and MDA exceeded the control values. After 7 days, the activity of liver GST in rats of the G4 was still high, and the activity of GPx and GR was lower, compared to rats of the G3. In addition, we found an inhibition of the activity of G6PDH (a key enzyme of the pentose cycle).

Discussion

The changes in the studied blood and liver parameters noted on the first day indicate the development of a stress reaction that caused an increase in glucose level in the blood serum due to increased breakdown of liver glycogen, the deficiency of which we noted during all periods of observation. The accumulation of lactate in the blood serum indicates an increase in the anaerobic oxidation of glucose by tissues, which leads to the activation of catabolism enzymes of purine mononucleotides.[15,16] As a result, an increased formation of uric acid in organs and tissues is developed. At the same time, xanthine oxidase produces free radicals,[16,17] which enhances the LP processes in cell membrane structures with the accumulation of MDA in them and contributes to the development of GSH deficiency due to GPx activation. Another factor contributing to the decrease in the GSH pool in liver cells is the activation of cypermethrin biotransformation systems (Figure 1), mainly due to GST. This enzyme promotes the formation of GSH conjugates with cypermethrin metabolites and LP products,[18] which are subsequently excreted from the body. Thus, tissues lose GSH and are forced to synthesize it *de novo* from amino acids with the energy expenditure of ATP. In addition, the activation of microsomal oxidation, which is also part of the xenobiotic biotransformation system, can indirectly contribute to the development of GSH deficiency.[19] An increase in the activity of microsomal oxygenase leads to the depletion of NADPH reserves, which is necessary for the formation of the reduced glutathione from GSSG.

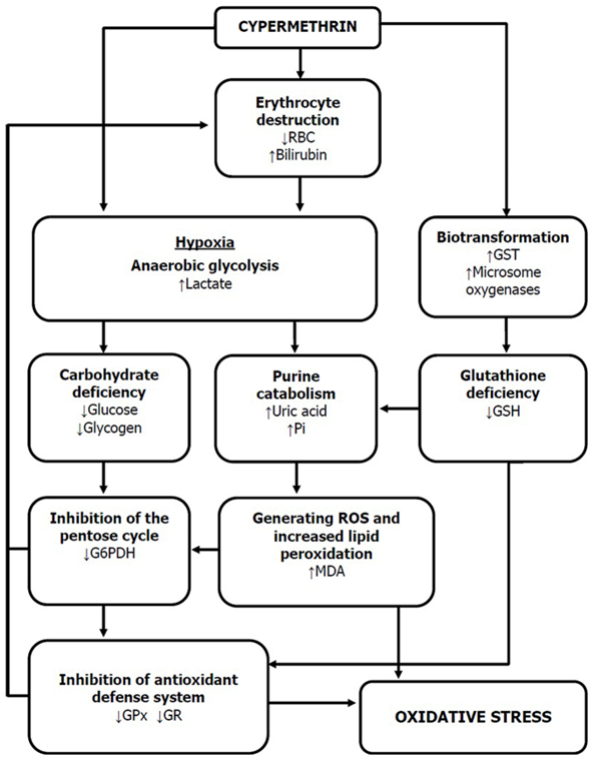

Fig. 1. *Triggers of oxidative stress during the action of cypermethrin on the body.*

Seven days after cypermethrin is administered to rats, glucose deficiency develops due to the depletion of glycogen stores in the liver and increased anaerobic oxidation. A decrease in RBC count enhances tissue hypoxia and further stimulates anaerobic glycolysis, leading to rapid and inefficient consumption of glucose and its conversion to lactate. The erythrocytes that are destroyed simultaneously release heme, which later turns into bilirubin, leading to its accumulation in the blood serum (Figure 1).

Carbohydrate deficiency and increased free radical oxidation in rats of the G4 lead to a decrease in the efficiency of the pentose cycle. This is evidenced by a decrease in G6PDH activity. This reduces the efficiency of the antioxidant system due to a reduction in the production of NADPH, which is necessary for the formation of the reduced glutathione from GSSG, and also leads to a deficiency of ribose-5-phosphate, which is necessary for the *de novo* synthesis of purines and their recycling. As a result, the metabolic shifts described above only increase. Suppression of G6PDH activity and a decrease in the effectiveness of the antioxidant system are the causes of damage to erythrocyte membranes and their hemolysis.

Conclusion

The triggers for the development of oxidative stress under cypermethrin exposure are lactic acidosis and increased catabolism of purine mononucleotides, accompanied by an increase in the production of free radicals and inhibition of the function of the antioxidant system. A decrease in the blood RBC, carbohydrate deficiency, and suppression of the activity of the pentose cycle 7 days after the administration of cypermethrin aggravate this condition.

Competing Interests

The authors declare that they have no competing interests.

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