

THE GLUTATHIONE REDOX SYSTEM ACTIVITY UNDER THE INFLUENCE OF ALPHA-CYPERMETHRIN IN RATS

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المخلص:

تتمثل هذه المعطيات في مستوى الجلوتاتيون و نشاط أهم أنزيمين مشاركين في تفاعلات الأكسدة الاختزالية للجلوتاتيون (الجلوتاتيون بيروكسيداز و الجلوتاتيون ريدوكتيز) في كبد الفئران المخبرية بعد ساعتين من المعاملة بـ 72 مغ/كغ (10/1 من LD_{50}) من المبيد الفاسييارمترين. حيث أوضحت النتائج المتحصل عليها بأن المعاملة بـ 10/1 من LD_{50} (عشر نصف الجرعة المميتة) من مبيد الفاسييارمترين أثرت على نظام الأكسدة الاختزالية للجلوتاتيون، بمعنى أن التعرض لمبيد الفاسييارمترين أدى إلى انخفاض في مستوى الجلوتاتيون الكبدي بنسبة 54 % ($P < 0.001$). نشاط الأنزيم جلوتاتيون بيروكسيداز و الذي يشارك في تفاعلات إزالة سمية المركبات السامة الناتجة عن تمثيل مبيد الفاسييارمترين زاد في الكبد بنسبة 50 % ($P < 0.05$). نفس الشيء يمكن ملاحظته في أنزيم الجلوتاتيون ريدوكتيز و الذي زاد من نشاطه بنسبة 47 % كذلك ($P < 0.001$). وبالرغم من الزيادة المعنوية التي ظهرت في نشاط الأنزيم إلا أنها لم تكن كافية للحفاظ على النسبة $GSH/GSSG$ طبيعية، نظرا للنقص الكبير في مستوى الجلوتاتيون الكبدي (بنسبة 54 %).

Summary:

Data are presented on the hepatic glutathione content and on the activity of two main enzymes involved in oxidoreduction reactions of glutathione (glutathione peroxidase and glutathione reductase) in the rat's liver treated with 72 mg/kg (1/10 of an LD_{50} dose) of a pesticide alpha-cypermethrin for 2 hours. The results indicate that the treatment with 1/10 of an LD_{50} of alpha-cypermethrin can affect the glutathione redox system. However, the exposure to alpha-cypermethrin resulted in a 54 % reduction ($P < 0.001$) in reduced hepatic glutathione. The glutathione peroxidase activity, which is involved in the detoxification of peroxide compounds arising from alpha-cypermethrin metabolites, increases by 50 % ($P < 0.05$). Similarly, the glutathione reductase increases by 47 % ($P < 0.001$). Despite the increased activity of glutathione reductase, it is not in correlate with the decrease in glutathione content (54 %) to maintain a stable ratio of $GSH/GSSG$.

Key words: Glutathione, Glutathione peroxidase, Glutathione reductase, Alpha-cypermethrin, Rats.

INTRODUCTION:

The alpha-cypermethrin (Alpha-cyan-3-phenoxybenzyl-1,3-dichlorovinyl-2-dimethyl chloropropane carboxylate) is a neurotoxicant synthetic pyrethroid that has been widely used as a pesticide and its LD_{50} for rat is estimated 720 mg/kg. The majority of these compounds are metabolised in

mammals and are readily excreted through the digestive apparatus or in the milk [1,2]. Their great lipophilicity makes them active toxic substances, which have an influence on the liver, kidneys etc. These organs are distinguished not only by their great lipid content but they are also organs of excretion and detoxification [2].

The glutathione defense enzyme system in living cells detoxifies and eliminates both

endogenous materials and xenobiotics, leading to the formation of products easily soluble in water and to their rapid elimination from the organism. These processes pass through many stages and interact closely with other enzymes, transforming compounds such as pesticides, drugs, hormones... etc, which are found in the organism, into non-toxic or less toxic products [3-5].

The glutathione redox cycle includes two main enzymes: glutathione reductase (GR) or [NADPH: oxidized glutathione oxidoreductase, EC: 1.6.4.2], which transforms the oxidized glutathione (GSSG) into its reduced form (GSH), and the glutathione peroxidase (GPx) or [glutathione:hydrogen-peroxide oxido-reductase, EC: 1.11.1.9], which activates the reaction of transforming the hydroperoxides into a primary alcohol. This enzyme system includes also the glutathione S-transferase (GST), which catalyses the reaction between the reduced glutathione and xenobiotic, carcinogenic and electrophilic compounds with the formation of GSH-conjugates [3]. The tripeptide glutathione is known for its important role in the detoxification of electrophilic toxic compounds and xenobiotics through conjugative reactions, catalysed by glutathione S-transferase with the formation of mercapturic acids [4,5]. The reduced glutathione is also involved in the detoxification of many toxic peroxides, by supplying these compounds with protons, transforming them into primary alcohols in the presence of glutathione peroxidase [6]. In the process of these detoxifying reactions the reduced glutathione (GSH) oxidizes into oxidized form (GSSG), so its level in the cells decreases, (see also fig 1).

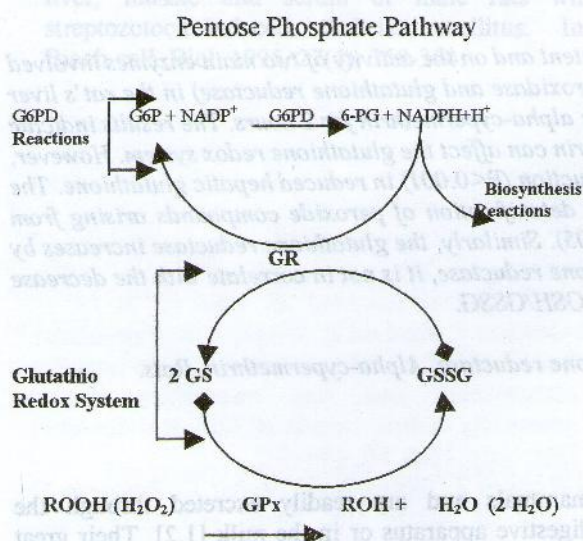


Figure 1: Physiological role of glutathione redox system, and its relationship with detoxifying process via oxido-reduction reactions of NADPH + H⁺.

The oxidation of reduced glutathione by glutathione peroxidase, which is connected with the

reduction of oxidized glutathione by glutathione reductase, is important in defining the redox status of glutathione. The regulation of GSH/GSSG is an important function for both enzymes and it is very high in detoxification organs such as liver, kidney, intestines, which sustains also the integrity of the cell membranes against peroxide effects [7,8].

The aim of this research was to study the glutathione redox system in the liver of male rats after their treatment with 1/10 of an LD₅₀ dose of alpha-cypermethrin. These investigations were in relation with the clarification of the metabolism of this pyrethroid pesticide in the liver as a site of detoxifying reactions in the organism.

MATERIALS AND METHODS

All chemicals used in this work were purchased from *Sigma Chemical Company*. Laboratory animals, albino Wistar male rats were brought from *Algiers Institute Pasteur*, at the age of 10 weeks, with an average live weight of 250 g. They were located in a room with an ambient temperature 21±1°C and 12 hours of light daily. They have a standard specified diet for rats and daily water renewal. The experimental animals were treated once by oral gavage (*per os*) with 85 mg/kg (1/10 of an LD₅₀ dose) of alpha-cypermethrin in the olive oil solution form. The control group is treated once *per os* with olive oil only. Two hours after treatment, the rats were killed by decapitation and the liver was taken out. Thirty-six liver organs were used in measurement of glutathione reductase and glutathione peroxidase activities and glutathione content.

The reduced glutathione was determined spectrophotometrically, according to the Weckbercker and Cory method [9]. The glutathione peroxidase activity was estimated by the Pinto and Bartley method [10], according to which the enzyme activity was evaluated by the amount of GSSG formed. The level of GSSG was measured depending on the method of Klotzch and Bergmeyer [11]. The glutathione reductase was determined by the Horn method [12], and the content of the protein by the method of Bradford [13]. Statistical analysis was made using Student's *t* test.

RESULTS AND DISCUSSION

The results show that the treatment of rats with 72 mg/kg (1/10 of an LD₅₀ dose) of alpha-cypermethrin causes a disturbance in the glutathione redox cycle, which leads to the appearance of toxic electrophilic and hydroperoxide compounds. The effect of the alpha-cypermethrin on the level of reduced glutathione in the liver cytosol is shown in table 1.

Table 1: Hepatic glutathione level (nM/mg prot) in control and treated rats with 72 mg/kg (1/10 of an LD₅₀ dose) of alpha-cypermethrin.

Sample Nb	Control group	Treated group
1	39.73	19.70
2	49.25	23.42
3	49.20	19.03
4	46.67	20.09
5	41.00	21.80
6	39.80	16.79
Means ± SD	44.27 ± 4.60	20.13 ± 2.28 ***

*** Significantly different ($P < 0.001$) from control group.

The data show a 54 % decrease of the content of reduced glutathione in the liver ($P < 0.001$) due to its inclusion in the detoxification processes of the active metabolites, caused by alpha-cypermethrin. The liver cells play an important role in the metabolism of pyrethroid through many reactions, producing toxic intermediary metabolites such as peroxides and xenobiotics. These compounds enter in oxido-reduction or conjugation reactions with the participation of reduced glutathione, as a result of this high consumption; its level decreases in the cells. The reduced glutathione will know for its important role in the bio-transformation of foreign exogenic substances including xenobiotics arising from pesticides [14,15], and lipid peroxides arising from heated oils and also with its function as a cell protector against their toxic action [16].

The decreased level of reduced glutathione is in accordance with the increased activity of the first enzyme in the glutathione redox cycle (glutathione peroxidase); it is concluded using the data from table 2. The activity of this enzyme is 52 % higher in alpha-cypermethrin treated liver cytosol than in the controls ($P < 0.05$).

Table 2: The activity of glutathione peroxidase (mU/mg prot) in liver cytosol of control and treated rats with 72 mg/kg (1/10 of an LD₅₀ dose) of alpha-cypermethrin.

Sample Nb	Control group	Treated group
1	0.900	1.375
2	0.785	1.510
3	0.905	1.563
4	1.130	1.250
5	0.790	1.071
6	1.008	1.572
Means ± SD	0.919 ± 0.13	1.390 ± 0.20 *

* Significantly different ($P < 0.05$) from control group

The capacity of reduced glutathione to reduce hydroperoxides, obtained under the influence of alpha-cypermethrin through GPx, leads to its oxidation into oxidized glutathione and this due to its decreased content. The increased activity of this enzyme (GPx) shows that the liver cells probably contain a high concentration of H₂O₂ and peroxides [17,18]. The degree of aerobic oxidation of glutathione has an effect on H₂O₂, hydroperoxides and glutathione peroxidase activity. Owing to the participation of H₂O₂ in the oxidation of reduced glutathione, it is possible that the catalase enzyme also takes a part in the reduction of H₂O₂. Through the oxido-reduction reaction of glutathione peroxidase, the reduced glutathione is capable to reduce and detoxifies the hydroperoxides, formed by metabolising reaction of the alpha-cypermethrin, where it oxidises to oxidized glutathione, it is due to the significant decrease of its level.

For the maintenance of a normal ratio of GSH/GSSG, which is approximately 100/1, glutathione reductase (as a second enzyme in the glutathione redox cycle) enters through oxido-reduction reaction by utilising NADPH+H⁺ for the reduction of oxidized glutathione to the reduced form. However the high diminution of the content of reduced glutathione under the influence of alpha-cypermethrin does not allow its level to raise, despite the increased activity of glutathione reductase with 47 % ($P < 0.001$). It is conjugated with the increased activity of glutathione peroxidase, which is 50 % higher for the alpha-cypermethrin-treated animals ($P < 0.001$) than for the control group (Table 3).

Table 3: The activity of glutathione reductase (U/mg prot) in liver cytosol of control and treated rats with 72 mg/kg (1/10 of an LD₅₀ dose) of alpha-cypermethrin.

Sample Nb	Control group	Treated group
1	2.23	3.27
2	2.36	2.97
3	2.18	3.21
4	1.86	3.29
5	2.06	3.07
6	1.92	2.82

Means ± SD 2.10 ± 0.19 3.10 ± 0.19 ***

*** Significantly different ($P < 0.001$) from control group.

The decreased level of glutathione, compared to the high activity of glutathione peroxidase shows that the reaction is greatly extended to the right, and is probably caused by the appearance of considerable quantity of peroxides resulting under

the influence of alpha-cypermethrin. The presence of glutathione reductase is essential for the live cell defense against hydroperoxides. Through it, the oxidized glutathione (GSSG) reduces into its reduced form (GSH).

CONCLUSION

In conclusion these findings show that the both enzymes in the glutathione redox system (glutathione peroxidase and glutathione reductase) have increased by 50 % and 47 % respectively after the treatment with 72 mg/kg (1/10 of an LD₅₀ dose) of alpha-cypermethrin, while the level of reduced glutathione has diminished to 54 %, due to it's participation in the elimination of toxic effects of alpha-cypermethrin.

Finally we can say that the alpha-cypermethrin as a synthetic pyrethroid, had a toxic effect on the liver, where it influences on the glutathione redox system, increases the formation of peroxides and inhibits the cellular glutathione biosynthesis.

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RESULTS AND DISCUSSION

The results show that the treatment of rats with 72 mg/kg (1/10 of an LD₅₀) of alpha-cypermethrin causes a disturbance in the glutathione redox cycle which leads to the appearance of toxic electrophilic and hydroperoxide compounds. The effect of alpha-cypermethrin on the level of reduced glutathione (GSH) and glutathione reductase (GR) is shown in Table 1. The results show that the treatment of rats with 72 mg/kg (1/10 of an LD₅₀) of alpha-cypermethrin causes a significant decrease in the level of reduced glutathione (GSH) and a significant increase in the level of glutathione reductase (GR).