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Effect of Alphamethrin on Glutathione Redox System in Rats

Aouacheri W., Saka Saad and Djafer R.
Department of Biochemistry, Faculty of Sciences, University of Badji, Mokhtar, Annaba, PO Box 12, Annaba 23000, Algeria

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Abstract. 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 85 mg/kg ($1/10^{th}$ of an LD_{50} dose) of an insecticide alphamethrin for 2 hours. The results indicate that the treatment with $1/10^{th}$ of an LD_{50} of alphamethrin can affect the glutathione redox system. However, the exposure to alphamethrin resulted in a 60% reduction ($P<0.001$) in reduced hepatic glutathione. The glutathione peroxidase activity, which is involved in the detoxification of peroxide compounds arising from alphamethrin metabolites, increases by 52% ($P<0.05$). Similarly, the glutathione reductase increases by 52% ($P<0.001$). Despite the increased activity of glutathione reductase, it is not in correlate with the decrease in glutathione content (60%) to maintain a stable ratio of GSH/GSSG.

***Keywords*:** Glutathione, Glutathione peroxidase, Glutathione reductase, Alphamethrin, Rats.

Introduction

Alphamethrin (alpha-cyan-3-phenoxybenzyl-1,3/2-dichlorovinyl-2-dimethyl chloro propane carboxylate) is a neurotoxicant synthetic pyrethroid that has been widely used as an insecticide and its LD_{50} for rat is estimated 850 mg/kg (Shell Company Ltd.). The majority of these compounds are metabolized 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 catalyzes 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, catalyzed 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 the oxidized form (GSSG), so its level in the cells decreases. For example, the content of reduced glutathione decreases by 35% for animals receiving 10 μg/kg buthionine sulfoximine [7] and by more than 50% for rats treated with 2.5 mg/kg of the synthetic oestrogen vitesterol [5].

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 ratio 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 [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 LD_{50} dose of alphamethrin. 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 Pasteur Institute, at the age of 10 weeks, with an average live weight of 200 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 ($1/10^{th}$ of an LD_{50} dose) of alphamethrin 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. Twelve 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 [14].

Results and Discussion

The results show that the treatment of rats with 85 mg/kg ($1/10^{th}$ of an LD_{50} dose) of alphamethrin causes a disturbance in the glutathione redox cycle, which leads to the appearance of toxic electrophilic and hydroperoxide compounds. The effect of alphamethin on the content of reduced glutathione and detoxifying enzymes in the liver cytosol are shown in Table 1.

The data show a 60% 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 alphamethrin. 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 [15, 16], and lipid peroxides arising from heated oils and also with its function as a cell protector against their toxic action [17].

The decreased level of reduced glutathione is in accordance with the increased activity of the first enzyme in the glutathione redox cycle (glutathione peroxidase). The activity of this enzyme is 52% higher in alphamethrin treated liver cytolsol than in the controls ($P<0.05$). The capacity of reduced glutathione to reduce hydroperoxides, obtained under the influence of alphamethrin through GPx, leads to its oxidation into oxidized glutathione and this is due to its decreased content. The increased activity of this enzyme (GPx) shows that the liver cells probably contain a high concentration of H_2O_2 and peroxides [18, 19]. The degree of aerobic oxidation of glutathione has an effect on H_2O_2, hydroperoxides and glutathione peroxidase activity. Owing to the participation of H_2O_2 in the oxidation of reduced glutathione, it is possible that the catalase enzyme also takes a part in the reduction of H_2O_2. Through the oxido-reduction reaction of glutathione peroxidase, the reduced glutathione is capable to reduce and detoxify the hydroperoxides, formed by the metabolizing reaction of alphamethrin, where it oxidizes to oxidized glutathione. As a result, a significant decrease of its level was noticed.

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 utilizing $NADPH+H^+$ for the reduction of oxidized glutathione to the reduced form. However, the high diminution of the content of reduced glutathione under the influence of alphamethrin does not allow its level to raise, despite the increased activity of glutathione reductase with 52% ($P<0.001$). It is conjugated with the increased activity of glutathione peroxidase, which is 52% higher for the alphamethrin-treated animals ($P<0.001$) than for the control group.

Table 1. Effect of alphamethrin on hepatic glutathione content and detoxifying enzymes in control and treated rats with 85 mg/kg ($1/10^{th}$ of an LD_{50} dose)

Glutathione content (nM/mg prot)		Glutathione peroxidase (mM/mg prot)		Glutathione reductase (U/mg prot)	
Control group	**Treated group**	**Control group**	**Treated group**	**Control group**	**Treated group**
49.25	17.70	0.900	1.385	2.23	3.47
39.73	21.41	0.785	1.530	236	2.94
46.67	17.03	0.905	1.583	2.18	3.25
49.20	18.05	1.130	1.252	1.86	3.39
39.80	17.71	0.790	1.078	2.06	3.17
41.00	15.64	1.008	1.588	1.92	2.95
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44.27 ± 4.60	17.92 ± 1.91	0.919 ± 0.13	1.402 ± 0.20	2.10 ± 0.19	3.19 ± 0.22

* Significantly different ($P < 0.05$) from control group.
*** Significantly different ($P < 0.001$) from control group.
1 : means ± SD.

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 quantities of peroxides resulting under the influence of alphamethrin. The presence of glutathione reductase is essential for the liver cell defense against hydroperoxides. Through it, the oxidized glutathione (GSSG) is reduced into its reduced form (GSH).

Conclusion

In conclusion, these findings show that glutathione reductase and glutathione peroxidase have increased by approximately 52% after alphamethrin treatment with 85 mg/kg (1/10 of an LD_{50} dose), while the level of reduced glutathione has diminished to 60%, due to its participation in the elimination of toxic effects of alphamethrin.

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

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تأثير مبيد الألفامثرين على نظام الأكسدة الأختزالية للجلوتاثيون عند الفئران

عواشري و.، سافع سعد، و جعفر ر.

قسم الكيمياء الحيوية، كلية العلوم، جامعة باجي مختار، عنابة،

ص.ب ١٢ ، عنابة ٢٣٠٠٠ ، الجمهورية الجزائرية

(قدم للنشر في ١٤٢٣/١٠/٢١هـ؛ وقبل للنشر في ١٤٢٦/٢/٦هـ)

ملخص البحث. تستهدف هذه الدراسة تقدير مستوى الجلوتاثيون ونشاط أهم أنزيمين مشاركين في تفاعلات الأكسدة الاختزالية للجلوتاثيون (الجلوتاثيون بيروكسيديز والجلوتاثيون رداكتيز) في كبد الفئران المخبرية بعد ساعتين من المعاملة بـ ٨٥ مج/كمج (١/١٠من LD_{50}) من المبيد ألفامترين.

أوضحت النتائج المتحصل عليها بأن المعاملة بـ ١/١٠ من LD_{50} (عشر الجرعة النصفية المميتة) من مبيد ألفامترين قد أثرت على نظام الأكسدة الاختزالية للجلوتاثيون. انخفض مستوى الجلوتاثيون الكبدي بنسبة ٦٠ ٪ ($P<0.001$) ، بينما زاد نشاط الأنزيم جلوتاتيون بيروكسيديز الذي يشارك في تفاعلات إزالة سمية المركبات السامة الناتجة عن أيض مبيد ألفامترين في الكبد بنسبة ٥٢٪ ($P<0.05$). الشيء نفسه حدث لأنزيم الجلوتاثيون رداكتيز الذي زاد من نشاطه بنسبة ٥٢٪ ($P<0.001$). وبالرغم من الزيادة المعنوية التي ظهرت في نشاط الأنزيم إلا أنها لم تكن كافية للحفاظ على النسبة الطبيعية بين GSH/GSSG . نظرا للنقص الكبير في مستوى الجلوتاثيون الكبدي(بنسبة ٦٠٪).

كلمات مفتاحية : جلوتاثيون، جلوتاثيون بيروكسيديز، جلوتاثيون رداكتيز، ألفامثرين، فأر.