Polish Journal of Environmental Studies Vol. 10, No. 4 (2001), 279-282

Organophosphate Insecticide Chlorfenvinphos Affects Superoxide Dismutase, Catalase and Malondialdehyde in Rat Liver

A. Łukaszewicz-Hussain

Department of Toxicology, Medical University of Bialystok,
Mickiewicza 2c, 15-222 Białystok, Poland

Received: October 26, 2000
Accepted: February 19, 2001

Abstract

The aim of this paper was to study the activity of liver superoxide dismutase and catalase and the concentration of liver malondialdehyde in rats intoxicated with chlorfenvinphos, an organophosphate widely used as an insecticide.

The study was carried out on male Wistar rats weighing 180-230 g. The rats were divided into two groups: examined - receiving oil solution of chlorfenvinphos in doses of 0.5 LD_{50}, and 0.1 LD_{50} and control group - receiving oil. The activity of superoxide dismutase (SOD), catalase (CAT) and concentration of malondialdehyde (MDA) were determined after 1, 24 and 48 hours of intoxication.

We observed an increase in the liver activity of SOD in further period of intoxication with chlorfenvinphos in both doses and a decrease of liver SOD activity in the rats intoxicated with the higher dose. The CAT activity in liver of the treated rats increased at the 1st hour of experiment with a dose of 0.5 LD_{50} and at the 1st hour and the 24th hour after intoxication with a dose 0.1 LD_{50}. Hepatic concentration of MDA showed a decrease at the 24th hour of intoxication with chlorfenvinphos and an increase at the 48th hour of intoxication with the higher dose and returned to control value for the rats intoxicated with the lower dose.

SOD and CAT play a major role in the maintaince of the physiological level of reactive form of oxygen. When reactive oxygen species generation exceeds capability of redox degrading systems, MDA levels increase.

The results obtained suggest that reactive oxygen species in liver injury might be caused by chlorfenvinphos.

Keywords: chlorfenvinphos, organophosphate insecticide, liver, acute poisoning, SOD, CAT, MDA

Introduction

Chlorfenvinphos (2-chloro-l (2,4-dichlorophenyl) vinyldiethyl phosphate) is an organophosphate, widely used singly or in mixture as an insecticide. Chlorfenvinphos, as other organophosphate compounds, is known to produce toxic effect by the inhibition of the acetylcholinesterase (AChE) activity.

In our ealier works we studied the effect of chlorfenvinphos on the activity of liver enzymes in the serum and in the liver homogenates and activity of serum cholinesterase - the marker of organophosphate poisoning [1, 2]. It was found that chlorfenvinphos led to an increase of acid hydrolases and glutamic-oxaloacetic and glutamic-pyruvic transaminases activity in the serum and decreased their activity in the liver. The degree of increase in the serum activity of cellular enzymes depended on the magnitude and severity of cell damage. The mechanism of this damage is not fully understood. The symptoms of glycolytic process enhancement and liver anoxia (reduced number of glycogen granules, high concentration of lactate and disminished level of pyruvate) were

observed in acute poisoning with chlorfenvinphos [3, 4]. Along with returning to the normal oxygen metabolism reactive oxygen species (ROS) are generated [5]. Reactive oxygen species are by-products of many normal physiological processes. When ROS generation exceeds capability of endogenous redox degrading systems, some pathophysiological events which lead to hepatocellular injury occur. The dominant sites of ROS production are mitochondria. They represent the major cellular compartment that consumes oxygen and produces energy by reduction of molecular oxygen to water in the electron transport system. Almost two percent of oxygen used by mitochondria is converted to superoxide radicals - the primary product of oxygen reduction [6]. The superoxide can cross the cell membrane via anion channels and can form more reactive radicals. The radicals increase membrane permeability and impair mitochondrial function.

In the present work the role of the disturbance of oxidative-antioxidative balance was investigated.

Materials and Methods

Animals

The studies were conducted on male Wistar rats of body weight of 180-230 grams.

The rats were fed standard diet and given water drink ad libitum.

The animals were divided into two groups: the control group, which received oil intragastrically by stomach tube in the amount of 0.1 ml/100 g, and the studied group, which received oil solution of chlorfenvinphos in doses of 0.1 LD_{50} and 0.5 LD_{50}.

Biochemical Determination

The rats were euthanized with ether (1, 24, and 48 hours after intoxication with chlorfenvinphos), then livers were quickly removed and placed in iced 0.9% NaCl containing 0.16 mg/ml heparin. Then the livers, for superoxide dismutase determination, were washed in ice cold 0.25 M sucrose, blotted on paper, weighed and homogenized in 9 vol. ice-cold sucrose. 10% homogenate centrifuged at 8500 g, 4°C for 10 minutes. An aliquot of 400 µl of ice-cold extraction reagent (ethanol/chloroform, 62.5/37.5 v/v) was added to 250 µl of supernatant and centrifuged at 3000 g for 10 minutes. Assay of SOD activity was performed with Bioxytech SOD-525TM Assay kit (OXIS International Inc., USA). The method is based on the SOD mediated increase in the rate of autooxidation of chromogen Rl in aqueous alkaline solution to yield a chromophore with maximum absorbance at 525 nm.

Catalase activity was measured in 10% liver homogenate prepared in phosphate buffer, centrifuged at 9000 g, 4°C for 15 minutes. The activity was determinated as described by Aebi [7]. Protein concentration was determined according to Lowry et al. [8], using bovine serum as a standard. Enzyme activities were expressed as units of enzyme activity per miligrams of protein.

Malondialdehyde concentration was determined as a thiobarbituric acid reactive substances in the liver homogenates in 0.15 M KCl according to Buege and Aust [9].

Statistical Analysis

The results were expressed as means ± SD. These results were analysed by standard statistical analyses, two-way Anova with Tukey's test for multiple comparisons to determine significance between different groups. The value for $p < 0.05$ was considered statistically significant.

Results

The activity of SOD in the livers of rats intoxicated with chlorfenvinphos a in dose 0.5 LD_{50} decreased at the 1st hour after treatment and increased at the 24th and 48th. When the compound was given in a dose of 0.1 LD50, activity of this enzyme did not change at the 1st hour in comparison to the control group and increased in the further periods of intoxication (Table 1).

The CAT activity increased in the rat liver at the 1st hour of intoxication with chlorfenvinphos in a dose of 0.5 LD_{50}, and at the 1st and 24th hour after intoxication with

Table 1. SOD activities (U/mg protein) in liver of rats intoxicated with chlorfenvinphos.

Control	Chlorfenvinphos					
	0.5 LD_{50}			0.1 LD_{50}		
	1h	24h	48h	1h	24h	48h
270.73 ± 26.59 n = 7	187.85 ± 38.15[a] n = 6	429.93 ± 64.25[ab] n = 6	397.32 ± 57.73[ab] n = 5	345.02 ± 105.9[b] n = 5	404.5 ± 134.27[ab] n = 5	386.14 ± 63.1[ab] n = 6

Legend:
values expressed as means ± SD
n - the number of rats in the group
p - statistically significant differences in comparison with: control - a, O.5LD_{50} - lh - b.

Table 2. CAT activities (U/mg protein) in liver of rats intoxicated with chlorfenvinphos.

Control	Chlorfenvinphos					
	$0.5\ LD_{50}$			$0.1\ LD_{50}$		
	1h	24h	48h	1h	24h	48h
103.50 ± 14.58 n = 6	133.48 ± 19.09^{a} n = 7	95.93 ± 15.35^{b} n = 7	117.63 ± 23.03 n = 6	182.82 ± 43.92^{abcd} n = 5	157.86 ± 35.28^{ac} n = 5	73.20 ± 18.25^{abcdef} n = 6

Legend:
values expressed as means ± SD
n - the number of rats in the group
p - statistically significant differences in comparison with: control - a, $0.5LD_{50}$-lh - b, $O.5LD_{5O}$-24h - c, $0.5LD_{5O}$-48h - d, $0.1LD_{50}$-lh -e, $0.1LD_{50}$-24h-f.

Table 3. MDA concentration (nmol/g tissue) in liver of rats intoxicated with chlorfenvinphos.

Control	Chlorfenvinphos					
	$0.5\ LD_{50}$			$0.1\ LD_{50}$		
	1h	24h	48h	1h	24h	48h
44.83 ± 7.25 n = 6	47.63 ± 9.78 n = 7	26.76 ± 1.20^{ab} n = 5	55.46 ± 2.72^{ac} n = 5	78.40 ± 16.10^{abcd} n = 6	30.20 ± 9.6^{abde} n = 6	52.20 ± 13.20^{cef} n = 7

Legend:
values expressed as means ± SD
n - the number of rats in the group
p - statistically significant differences in comparison with: control - a, O.5 LD_{50} lh - b, $0.5LD_{50}$-24h - c, $0.5LD_{50}$-48h - d, $0.1LD_{50}$-lh -e, $0.1LD_{50}$-24h-f.

insecticide in a dose of 0.1 LD_{50}. The highest CAT activity was observed at the 1st hour of intoxication with chlorfenvinphos in the lower dose, in which the CAT activity was decreased after 48 hours. The activity of this enzyme after 48 hours of poisoning was lower in comparison to the control group and in comparison to the other examined groups (Table 2).

Hepatic concentrations of MDA decreased 24 hours after intoxication with chlorfenvinphos at the higher dose and increased on the 48 hour. Concentration of MDA increased almost twofold at the 1st hour, decreased on the 24th and returned to the control value at the 48th hour in the livers of the lower dose treated rats (Table 3).

Discussion of Results

In the present study we demonstrated that chlorfenvinphos influences antioxidative enzymes and lipids peroxidation in liver and, therefore, liver injury associated with this insecticide may be due, at least in part, to oxidative tissue damage.

There is little evidence of change in antioxidant system after intoxication with organophosphate. Hai et al. [10] observed an increase of SOD and CAT activities in the liver of DDVP (dichlorvos)-treated carps. He also found elevated levels of MDA in the livers of DDVP-treated fish. Yang et al. [11] suggested that the organophosphates and carbamates, besides its inhibitory effect on acetylcholinesterase, initiates the accumulation of free radicals leading to lipid peroxidation (enhancement of MDA level). Bagchi et al. [12] observed increases in the levels of liver and brain MDA after treatment of rats with selected polyhalogenated cyclic hydrocarbons and organophosphate insecticides.

Antioxidant protection consists of many enzymatic and non enzymatic factors which maintain the physiological level of reactive forms of oxygen [13]. The antioxidant-defence system includes superoxide dismutase, catalase and glutathione peroxidase enzymes.

We determined the activity of two antioxidative enzymes: superoxide dismutase and catalase. The determination of MDA level in liver homogenates allows us to estimate the rate of cell membrane lipid peroxidation.

Superoxide dismutase plays a major role in the first line of the antioxidant defence system by catalyzing the dismutation of superoxide radical to form hydrogen peroxide and molecular oxygen [14]. Mechanism of catalyse provided by SOD suggests, that this enzyme is incomplete antioxidant which prevents the superoxide anion and produces the other. Its biological action is connected with catalase via H_2O_2. Catalase is a ubiquitous enzyme present in cells of aerobic organism. Catalase converts two molecules of the strong oxidant, hydrogen peroxide, to molecular oxygen and two molecules of water [15]. Kono et al. [16] found that superoxide anion inhibited catalase action and the presence of hydrogen peroxide inhibited the action of dismutase.

In our earlier studies we found an increase of lactate

concentration and a decrease of pyruvate level in the liver of rats intoxicated with chlorfenvinphos. The ratio lactate/pyruvate - an index of anaerobic glycolyse increased and glycogen granules disappeared in acute poisoning by this insecticide. The enhancement of the anaerobic process was dose-dependent [3, 4].

Tsan [17] found an increase of SOD activity in hypoxia. Chlorfenvinphos treatment increased the superoxide dismutase activity but only at the 24th and 48th hour after intoxication. Superoxide dismutase reached maximum, when MDA concentration decreased. From our earlier studies and from the above-cited paper [17] we suppose that changes in the hepatic SOD activity might be due to anoxia. Decreased levels of MDA confirms this suggestion (24 hours after intoxication).

In contrast to SOD, the activity of the hepatic catalase increased following chlorfenvinphos treatment of the higher dose only at the 1st hour and at the 1st and 24th hours after treatment of the lower dose. Enhanced activity of hepatic antioxidative enzymes, such as superoxide dismutase and catalase, would result in decreased levels of lipids peroxidation index (MDA). In the present study, however, enhanced activity of antioxidative enzymes was not necessarily related to reduction of MDA levels. In other words, the MDA level was elevated at the 1st hour of intoxication of lower dose when CAT activity reached maximum. Peroxidation of lipids occurs when prooxidant substances react with unsaturated fatty acids of biological membranes. The increase in MDA level indicates an enhancement of hepatic lipid peroxidation at the further period of intoxication with chlorfenvinphos of the higher dose. These results suggest the possibility of reoxidation process at the 48th hour of intoxication with chlorfenvinphos of the higher dose.

In this study we observed a decrease in the hepatic CAT acivity at the 48th hour of intoxication with chlorfenvinphos of lower dose. The decrease in CAT activity indicates a reduced ability to protect against hydrogen peroxides, whereas increased SOD activity indicates that protection against the superoxide radical is more required. These results suggest a lowered ability of the liver of rats intoxicated with chlorfenvinphos of lower dose (48 hours after treatment) to protect against oxidative damage. This suggestion is confirmed by increased levels of MDA. Thus, acute intoxication of rats with chlorfenvinphos caused disturbances in oxidative-antioxidative balance in the liver.

The interpretation of these results will be more complete after further investigation of other antioxidative enzymes and nonenzymatic antioxidative substances.

References

1. LUKASZEWICZ-HUSSAIN A., MONIUSZKO-JAKONIUK J., GALAZYN-SIDORCZUK M., The changes in activity of aminotransferase in serum and liver homogenate after poisoning with chlorfenvinphos. Bromat. Chem. Toksykol. 3, 279, 1996 (in Polish).
2. LUKASZEWICZ-HUSSAIN A., MONIUSZKO-JAKONIUK J., Activity of lysosomal enzymes in acute intoxication with organophosphorus insecticides. Pol. J. Environ. Studies 6, 51, **1997.**
3. LUKASZEWICZ-HUSSAIN A., MONIUSZKO-JAKONIUK J., Glycolytical process in the rat liver in chlorphenvinphos intoxication. Med. Pracy 48(5), 579, 1997 (in Polish).
4. LUKASZEWICZ-HUSSAIN A., CHYCZEWSKI L., MONIUSZKO-JAKONIUK J., Concentration of lactate and glucose in blood serum and of glycogen in liver in acute poisoning with chlorfenvinphos. Bromat. Chem. Toksykol. 31(3), 251, 1998 (in Polish).
5. SCHAPER W., SCHAPER J., Molecular mechanism in "stunned" myocardium. Cardiovasc. Drugs Ther. 5, 925, **1991.**
6. BOVERIS A., CHANCE B.,The mitochondrial generation of hydrogen peroxide: general properties and the effect of hyperbari oxygen Biochem. J., 133, 707, 1973.
7. AEBI H.E., Catalase in vitro. Methods Enzymol.105, 121, **1984.**
8. LOWRY O.H., ROSENBROUCH N.J., FARR A.L., RANDALL R.J., Protein measurement with Folin phenol reagent. J. Biol. Chem. 153, 267,1951 .
9. BUEGE J.A., AUST S., Microsomal lipid peroxidation. Methods Enzymol. 51, 302,1978.
10. HAI D. Q., VARGA SZ. I., MATKOVICS B., Organophosphate effects on antioxidant system of Carp (Cyprinus carpio) and Catfish (Italurus nebulosus). Comp. Biochem. Physiol. 117C(1), 83, 1997.
11. YANG Z.P., MORROW J., WU A., ROBERTS L.,J., DETTBARN W.D., Diisoprppylphosphorofluorodate-induced muscle hyperactivity associated with enhanced lipid peroxidation in vivo. Biochem. Pharm. 52, 357, 1996.
12. BAGCHI D., BAGCHI M., HASSOUN E.A., STOHS S.J., In vitro and in vivo generation of reactive oxygen species, DNA damage and lactate dehydrogenase leakage by selected pesticides. Toxicol. 104 (1-3), 129, 1995.
13. ONDREICKA R., BENO I., CERNA O., GRANICOVA E., STARUCHOWA M., VOLKOVA K., BOBEK P., TATARA M., Relationship between levels of vitamin C, E, A and beta-carotene and activity of antioxidant enzymes in the blood. Bratisl. Lek. Listy 99, 250, 1998.
14. HO Y.SH.,GARGANO M., CAO J., BRONSON R.T., HEIMLER I., HUTZ R.J., Reduced fertility in female mice lacking copper-zinc superoxide dismutase. J. Biol. Chem. 203(13), 7765, 1998.
15. LASZLO A, MATKOVICS B., VARGE SZ.I., WITTMAN T., FAZEKAS T., Changes in lipid peroxidation and antioxidant enzyme activity of human red blood cells after myocardial infarction. Clin. Chim. Acta. 203, 413, 1991 .
16. KONO Y., FRIDOVICH I, Superoxide radical inhibits catalase. J.Biol.Chem. 257,5751, 1982.
17. TSAN M.F., Superoxide dismutase and pulmonary oxygen toxicity.P.S.E.B.M.203, 286, 1993.