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Letter to the Editor

How Chlorfenvinphos Affects Serum Concentrations of Transition Metals, Hydrogen Peroxide and Erythrocyte Activity of Superoxide Dismutase

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Abstract

The aim of this paper was to examine the effects of chorfenvinphos on serum concentrations of transition metals, hydrogen peroxide and malondialdehyde (a lipid peroxidation index), and on the activity of superoxide dismutase in erythrocytes.

Male Wistar rats were treated with vehicle or 0.02, 0.1 and 0.5 x LD50 of chlorfenvinphos and samples were collected at 1, 24 and 48 hr after treatment. The experiments were approved by the Local Ethics Committee.

We demonstrated a decreased concentration of copper serum, which is accompanied by the increased activity of superoxide dismutase. The changes observed in the concentrations of copper can be explained by its displacement from serum to erythrocytes. We also observed increased levels of zinc serum (after intoxication with CVP at doses of 0.02 and 0.1 x LD_{50}) and iron, as well as enhancement in hydrogen peroxide serum and malondialdehyde concentration. The changes in serum Zn concentration probably resulting from cellular membrane damage and the increase in serum iron concentration, is probably caused by its release from haemoglobin. The changes of serum Fe levels seems to have no effect on lipid peroxidation.

We concluded that in acute intoxication with chlorfenvinphos — organophosphorus insecticide, the non-cholinesterase mechanismes are involved.

keywords: transition metals, H_2O_2, superoxide dismutase, chlorfenvinphos

Introduction

Chlorfenvinphos is an organophosphate compound used alone or in combination [1]. In our previous studies and in reports of other authors it has been demonstrated that this compound inhibits the activity of cholinesterase, the enzyme indicative of exposure to organophosphate insecticides, and leads to liver injury [1, 2]. The mechanism of liver damage, despite numerous studies, has not been fully elucidated. The involvement of reactive oxygen species in toxicity of these compounds has been suggested [3, 4, 5]. The so-called transition metals, such as iron, copper and zinc, play a significant role in the production of reactive oxygen species. Their ions can be found at different stages of oxidation, except for zinc, which is always associated with the second stage of oxidation. They can also transport electrons onto many biologically important molecules, including lipids, proteins and nucleic acids [6, 7, 8]. Additionally, iron and copper act as catalysts in the decomposition of organic peroxides into radicals [7].

Under normal conditions ions of transition metals are chelated by appropriate proteins and stored. However, even a slight decrease in pH accelerates the release of protein-bound transition metal ions, such as Fe (II) and

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Cu (II), and may increase superoxide anion generation [9, 10, 11]. In intoxication with organophosphate insecticides, pH becomes reduced [12]. In our earlier studies we observed an increase in serum concentration of lactate in rats receiving chlorfenvinphos in single doses [13]. Moreover, both copper and zinc are indispensable to the proper action of cytosolic superoxide dismutase [8].

Taking the above into consideration, we examined the effect of chorfenvinphos on serum concentrations of transition metals, hydrogen peroxide and malondialdehyde — a lipid peroxidation index, and on the activity of superoxide dismutase in erythrocytes.

Material and Methods

The study used male Wistar rats of 180-200 g body weight. The animals had free acccess to water and standard diet and maintained ad libitum. They were divided into the control group, which received olive oil by stomach tube, and experimental groups given oil solutions of chlorfenvinphos in doses of 0.02, 0.1 and 0.5 LD_{50} (LD_{50} = 15 mg/kg b. w.). The experiments were approved by the Local Ethics Committee.

Material for analysis was collected 1, 24 and 48 hours after intoxication. Blood serum concentrations of iron, zinc and copper were determined using the atomic-absorptive spectrophometry method with atomization in the air/acetylene flame (Hitachi 2000). Zinc concentration was assayed at a wavelength of 213.9 nm, copper at 324.7 nm and iron at 248.8 nm. The concentrations were expressed in mg/100 cm^3 serum. The activity of superoxide dismutase (SOD, EC 1.15.1.1) in erythrocytes and serum concentration of hydrogen peroxide were determined according to the colorimetric method using ready-made "Bioxytech" diagnostic kits (from Oxis International, Inc. OR, USA). The SOD assay method is based on the autooxidation rate of tetracyclic catecholes by SOD. The activity was expressed in units per cm^3 (U/cm^3). The assay of hydrogen peroxide is based on the formation of colour complex between iron ion and an indicative compound, which is xylene orange in this method. Serum concentration of hydrogen peroxide was expressed in $\mu mol/dm^3$.

Thiobarbituric acid and malondialdehyde (MDA) concentration was expressed as $\mu mol/dm^3$ and assayed according to the method of Buege and Aust [14].

The results were subjected to statistical analysis using the Mann-Whitney test. Differences were considered statistically significant at $p<0.05$.

Results

Serum iron concentration increased statistically significantly compared to control in acute intoxication with chlorfenvinphos, with the maximum increase at 48h (177% of control value) and at a dose of 0.5 x LD_{50} (Table 1). At 0.1 LD_{50} (app. 140% of control) and 0.02 LD_{50} (app.

Table 1. Level of iron, zinc and copper (μg/100ml) in the serum after acute intoxication with chlorfenvinphos.

	Fe	Zn	Cu
control	221.27±32.52 (n=11)	103.46±7.29 (n=10)	136.00±14.20 (n=11)
CVP-0.02LD_{50} *1 h*	287.23±19.46 [a] (n=8)	149.12±23.15 [a] (n=8)	118.54±10.12 [a] (n=8)
24 h	302.36±37.90 [a] (n=8)	140.12±21.15 [a] (n=8)	119.13±11.10 [a] (n=8)
48 h	308.99±51.11 [a] (n=8)	120.21±16.23 [a] (n=8)	132.13±12.12 (n=8)
CVP-0.1LD_{50} *1 h*	300.50±50.51 [a] (n=8)	151.14±28.25 [ad] (n=7)	106.79±10.66 [ad] (n=8)
24 h	263.71±43.32 [ad] (n=8)	132.43±24.66 [a] (n=6)	109.07±14.07 [ad] (n=6)
48 h	347.00±60.74 [af] (n=8)	125.83±14.97 [ae] (n=7)	105.45±11.40 [ad] (n=6)
CVP-0.5LD_{50} *1 h*	350.60±52.62 [abdef] (n=8)	87.40±8.73 [abcdefg] (n=6)	110.34±10.10 [a] (n=6)
24 h	326.60±16.98 [abdf] (n=8)	80.00±9.27 [abcdefg] (n=6)	88.07±8.16 [abcdh] (n=6)
48 h	394.00±50.51 [adfi] (n=8)	80.20±4.60 [abcdefg] (n=6)	107.46±7.15 [ad] (n=6)

value expressed as mean ± SD

statistically significant in comparison with: a- control, b- 0.02 LD_{50}-1h, c-0.02 LD_{50}-24h, d- 0.02 LD_{50}- 48h, e- 0.1 LD_{50}-1h, f- 01 LD_{50}-24h, g- 01LD_{50}-48h, h- 0.5LD_{50}- 1h, i-0.5LD_{50}-24 h.

151% of control, at 1 hr and 132% at 24 hr) an increase in serum zinc concentration was observed throughout the experiment. The changes were statistically significant compared to control (Table 1). The increase in Zn concentration, observed at 48h (app. 120% of control) following chlorfenvinphos administration at these doses, was not so high as that noted in the other time intervals. A fall in serum Zn concentration (app. 80% of control value) was observed during the whole examined period after intoxication with a dose of 0.5 LD_{50}. Copper concentration in the experimental groups was reduced (65% to 85%) statistically significantly, compared to the control group (Table 1). The reduction, however, was not found at 48h at the lowest dose, when the Cu concentration returned to the control level.

Chlorfenvinphos administration (0.02 LD_{50}) resulted in an increase (143% of control) in erythrocyte SOD activity initially (within 1hr) and the activity returned to control value by 24 hr (Table 2). In erythrocytes of rats given 0.1 LD_{50}, SOD activity was enhanced (app, 150% of control value) throughout the experiment. The values were higher not only in comparison to control but also to the values observed in animals receiving a higher dose. SOD activity in erythrocytes, after chlorfenvinphos administration at 0.5 LD_{50} increased statistically significantly compared to control only at 1 and 24 h (120% of control) after intoxication, and decreased at 48 h (app. 80% of control).

MDA serum concentration was significantly altered following CVP intoxication (120-180% of control) throughout the experiment. The values obtained at 1 and 24 h after intoxication with lowest dose were statistically higher compared to MDA levels observed after the higher doses (Table 2). Serum hydrogen peroxide concentration increased statistically significantly at 1h and 24h at the two lower doses and throughout the experiment at the highest dose of chlorfenvinphos (app. 140% of control), compared to control (Table 2).

Discussion

We evaluated the effects of CVP intoxication of serum levels of transition metals in the present study. The ions of transition metals are released from proteins and get involved (except of zinc) in the production of free oxygen radicals via catalysis of the Haber-Weiss reaction, which leads to the formation of highly reactive hydroxyl radical derived from superoxide anion radical or hydrogen peroxide [15]. Iron is the most commonly investigated and the most dangerous transition metal, whose ions can be relatively easily released from transferrin and ferritin [6, 8, 16, 17]. Free iron ions belong to the most powerful oxidative agents in the cell [16]. Even a slight increase in the concentration of free iron ions, unbound with proteins, leads to the production of

Table 2. Superoxide dismutase activity in the erythrocyte (U/ml), malondialdehyde (µmol/l) and hydrogen peroxide (µmol/l) level in the serum of rats after acute intoxication with chlorfenvinphos.

	SOD	MDA	H_2O_2
control	226.29±16.11 (n=12)	3.33±0.58 (n=15)	22.08±3.63 (n=10)
CVP-0.02LD_{50} *1 h*	323.96±28.84 ^{a} (n=8)	6.14±1.10 ^{a} (n=8)	27.83±1.52 ^{a} (n=8)
24 h	235.79±14.38 ^{b} (n=8)	5.62±0.89 ^{ab} (8)	29.31±1.04 ^{a} (n=8)
48 h	238.51±21.02 ^{b} (n=9)	4.20±0.37 ^{abc} (n=6)	23.63±2.63 ^{bc} (n=6)
CVP-0.1LD_{50} *1 h*	353.73±9.50 ^{acd} (n=7)	4.08±0.46 ^{abc} (n=9)	29.97±3.71 ^{a} (n=8)
24 h	358.78±19.90 ^{acd} (n=8)	4.86±0.59 ^{abc} (n=8)	26.93±1.12 ^{a} (n=8)
48 h	355.90±21.34 ^{acd} (n=7)	4.51±0.20 ^{abc} (n=8)	24.29±5.24 (n=8)
CVP-0.5LD_{50} *1 h*	279.26±12.01 ^{aefg} (n=7)	4.95±0.27 ^{abc} (n=7)	30.69±3.41 ^{ad} (n=8)
24 h	276.71±23.27 ^{abefg} (n=8)	4.18±0.23 ^{abch} (n=6)	29.45±1.79 ^{adfgh} (n=8)
48 h	177.01±20.56 ^{abcdfghi} (n=8)	4.72±0.74 ^{abc} (n=6)	27.74±3.83 ^{ad} (n=8)

acute intoxication with chlorfenvinphos value expressed as mean ± SD

statistically significant in comparison with: a- control, b- 0.02 LD_{50}-1h, c-0.02 LD_{50}-24h, d- 0.02 LD_{50}- 48h, e- 0.1 LD_{50}-1h, f- 01 LD_{50}-24h, g- 01LD_{50}-48h, h- 0.5LD_{50}- 1h, i-0.5LD_{50}-24 h.

free radicals and damage to the cellular membranes and DNA [18, 19, 20].

We found an increase in serum iron concentration in chlorfenvinphos intoxication. The increase was accompanied by a temporary rise in hydrogen peroxide and malondialdehyde levels. Studies in vitro on erythrocytes suggest that the increase in hydrogen peroxide level as well as lipid peroxidation lead to haemolysis of erythrocyte [21]. Iron release may be caused by hem degradation. "Free" iron can initiate the formation of hydroxyl radicals by the Fenton reaction [22]. A rise in hydrogen peroxide concentration may cause an increase in serum iron levels as suggested by others [21]. Our study has been demonstrated that an increase in iron level is accompanied by a temporary rise in hydrogen peroxide level. Based on the present results it is difficult to determine whether the changes in iron and hydrogen peroxide levels contribute to the increased production of highly reactive hydroxyl radical as postulated earlier [22]. The intensification of lipid peroxidation and the increase in MDA concentration occurred as well at the lowest dose as at the higher doses of chlorfenvinphos (0.1 LD_{50} and 0.5 LD_{50}). However, after intoxication with the lowest dose the highest value of serum MDA was observed at 1 and 24h. After intoxication with the dose of 0.5 LD_{50} the tendency to further increase at 48h was observed. By evaluating serum MDA behaviour in chlorfenvinphos intoxication it can be assumed that the MDA as well as hydrogen peroxide concentration probably influence the generation of reactive oxygen species in the skeletal muscles, as has been observed by other authors [23].

Copper and zinc, like iron, are also indispensable in the organism [6, 7, 24]. Copper is included in many enzymes, such as ceruloplasmin, cytochrome oxidase, or like zinc, in copper-zinc dismutase. In the organism, copper is transported by ceruloplasmin, albumin, transcuprein and in trace amounts by various amino acids. It is not yet clear how this metal is transported to the cell [25]. Zinc included in proteins is directly involved in chemical catalyses and is essential in the maintenance of the structure and stability of protein [26]. The biochemical function of zinc is the maintenance of cellular membrane structure and function [27, 28].

Copper in the form of free ions, like iron, catalyses reactions, leading to the production of free radicals, and also catalyses membrane lipid peroxidation [6, 29]. It is the major agent responsible for peroxidative modifications of lipoprotein fractions LDL [30]. On the other hand, copper is part of the antioxidative enzyme, i. e. superoxide dismutase. Zinc, however, plays a protective role in the systems that form reactive oxygen species through a mechanism involving the protection of sulfhydryl group oxidation [31]. In the reactions with chelating compounds, zinc ions compete against iron and copper, but it is not involved in the Fenton reaction [31]. This element acts as an antioxidant only at high concentrations.

In chlorfenvinphos intoxication, changes were observed in serum copper and zinc concentrations. At lower doses the increase in zinc concentration was accompanied by a decreased copper level. After administration of chlorfenvinphos at the highest dose, Zn concentration decreased and Cu level was still low. After intoxication with the lower doses of chlorfenvinphos, since zinc serum concentration was rapidly increasing, as was the MDA level, it may be assumed that the increase in zinc concentration is caused by damage to cellular membranes as an effect of peroxidation. Unlike the zinc level, copper concentration in serum was decreasing. In chlorfenvinphos intoxication the reduction in copper concentration was accompanied by increased SOD activity in erythrocytes. Except the 48h after intoxication with the highest dose, when a low level of copper was accompanied by a decrease in SOD activity. Thus it is assumed that the reduction in serum copper concentration results from increased demand for this element in erythrocytes, where it can be used to enhance SOD activity, but it is only indirect evidence because we did not determine copper concentration in erythrocytes in the present study.

Literature data indicate that the normal relationship between the level of copper and zinc is essential to regulate the production of interleukin, being an inflammation mediator [8]. In acute chlorfenvinphos intoxication this relationship was disturbed. We could not find any literature data concerning the relationship between these metals in the intoxication with organophosphate insecticides.

We found only one paper in the available literature in which zinc and copper concentrations were determined in the intoxication with chlorpyriphos, an organophosphate compound [32]. In chronic intoxication with this compound, concentrations of copper, iron and selenium increased in liver, while zinc concentrations decreased in liver and serum and copper in serum [32]. The latter changes were normalized in the groups receiving additional Zn in drinking water. The authors believe that zinc mediates toxic effects caused by chlorpyriphos. However, our experiments seem to indicate that the increase in zinc serum concentration is rather a toxic effect of chlorfenvinphos (damage to cellular membranes). The results of both studies, however, cannot be compared because different experimental models were used in these studies.

In conclusion, the displacement of copper from serum to erythrocytes in order to increase erythrocytes SOD activity probably caused the decrease of serum Cu level. In acute chlorfenvinphos intoxication there also occurs an increase in the serum level of hydrogen peroxide and alterations in malondialdehyde concentration. Thus, we demonstrated the non-cholinesterase action of organophosphorus insecticide — chlorfenvinphos.

In further study, clearly aiming to explain the relationship between erythrocytes SOD activity and changes in Cu concentration, we are going to examine the erythrocyte Cu level.

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