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Liver Catalase, Glutathione Peroxidase and Reductase Activity, Reduced Glutathione and Hydrogen Peroxide Levels in Acute Intoxication with Chlorfenvinphos, an Organophosphate Insecticide

A. Łukaszewicz-Hussain*, J. Moniuszko-Jakoniuk

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

Abstract

The aim of this study was to determine the effect of acute intoxication with chlorfenvinphos on the liver reduced glutathione (GSH) level and the activities of two enzymes involved in glutathione metabolism, as well as the activity of catalase (CAT), glucose-6-phosphate dehydrogenase (G6PDH) and the level of liver hydrogen peroxide. The concentration of malondialdehyde (MDA) was determined as lipid peroxidation index. Rats were divided into two groups: the control group, which received oil intragastrically by stomach tube, and the experimental groups, which received oil solution of chlorfenvinphos (CVP) in doses of 0.02 LD_{50}, 0.1 LD_{50} or 0.5 LD_{50}. After 1, 24, 48 hours of intoxication with chlorfenvinphos, the livers were quickly removed and placed in iced 0.9% NaCl containing 0.16 mg/ml heparin. The liver glutathione peroxidase (GPx) and reductase (GR) activities as well as GSH and hydrogen peroxide levels were determined using Bioxytech Assay kits. CAT activity was determined by Aebi method, G6PDH activity was measured using Sigma Assay kit. MDA level was determined by Buege and Aust method.

The changes of examined parameters of antioxidative system as well as lipid peroxidation index were found. The correlation between MDA concentration and the elements of enzymatic and non-enzymatic antioxidative system was determined. A statistically significant correlation was found only between MDA level and G6PDH activity.

In conclusion, it is suggested that G6PDH play a key role in the defence against oxidative stress induced by intoxication with chlorfenvinphos.

Keywords: chlorfenvinphos, antioxidative enzymes, glutathione, MDA

Introduction

Chlorfenvinphos is an organophosphate insecticide widely used alone or in combination in Poland. In our earlier works, we demonstrated changes in the activities of liver enzymes such as: acid phosphatase, β-glucuronidase, aminotransferases in the serum and liver of rats intoxicated with this compound [1, 2]. We also observed increased lactate levels in the livers and in the serum of intoxicated rats, as well as a decreased hepatic pyruvate level [3]. This study demonstrated the liver injury in the acute intoxication of rats with chlorfenvinphos. A number of other authors described the changes in the liver parameters of animals treated with some organophosphate insecticides, but the mechanism of this liver damage still remains not fully understood.

Experimental data show no correlation between liver damage and the degree of organophoshate induced acetylcholinesterase inhibition, the main mechanism of its toxicity, suggesting the involvement of alternative mechanisms.

*Corresponding author; e-mail: toxic@amb.edu.pl

Reactive oxygen species are known mediators of different origin injury of the liver as well as other organs. Some reports have suggested that reactive oxygen species (ROS) are involved in liver disturbances after treatment with organophosphate insecticides [4, 5].

The aim of this study was to determine the effect of acute intoxication with chlorfenvinphos on liver GSH levels and the activities of two enzymes involved in glutathione metabolism, as well as the activity of CAT and G6PDH and the level of liver hydrogen peroxide. The concentration of MDA was determined as lipid peroxidation index.

Material and Methods

The experiments were conducted on male Wistar rats of 200±20g of body weight. The rats were fed a standard diet and given water to drink ad libitum.

The animals were divided into two groups: the control group, which received oil intragastrically by stomach tube in the amount of 0.1ml/100g, and the experimental groups, which received oil solution of chlorfenvinphos i.e. 2-chloro-1-(2,4-dichlorophenyl) vinyldiethyl phosphate (CVP) in doses of 0.02 LD_{50}, 0.1 LD_{50} or 0.5 LD_{50} (LD_{50}=15 mg/kg b.w.).

1, 24, 48 hours after intoxication with chlorfenvinphos, the rat livers were quickly removed and placed in iced 0.9% NaCl containing 0.16 mg/ml heparin. Our study was approved by the Local Ethics Committee.

Catalase activity was measured in 10% liver homogenates prepared in phosphate buffer, centrifuged at 9000xg, 4°C for 15 minutes. The activity was determined as described by Aebi [6]. Glutathione peroxidase (GPx) was assayed in the liver sample homogenized in 8 volumes of cold buffer (50mM Tris-HCl, pH 7.5, containing 5 mM EDTA and 1 mM 2-mercaptoethanol), next centrifuged 8500xg for 10 minutes at 4°C. GPx activity was determined in supernatant using BIOXYTECH GPx-340™ Assay kit produced by OXIS International, Inc., USA. The GPx assay was based on the oxidation of NADPH to $NADP^+$, which is accompanied by a decrease in absorbance at 340nm. The rate of this decrease is directly proportional to the GPx activity in the sample. The liver for glutathione reductase (GR) determination was homogenized in cold buffer (50mM potassium phosphate, pH 7.5, containing 1mM EDTA) and then centrifuged at 8500xg for 10 minutes. The assay of GR activity was performed in supernatant using BIOXYTECH GR-340™ Assay kit produced by OXIS International, Inc., Portland, USA. The method is based on the oxidation of NADPH to $NADP^+$ catalysed by glutathione reductase. One molecule of NADPH is consumed for each molecule of reduced GSSG form. The reduction in GSSG is determined indirectly by the measurement of NADPH consumption, as demonstrated by a decrease in absorbance at 340nm as a function of time. G6PDH activity was measured in 10% liver homogenate prepared in ice-cold 0.9% NaCl solution, centrifuged at 8500g, 4°C for 15 minutes. The activity was determined in supernatant using Sigma Diagnostic kit, Inc., St. Louis, USA. The method is based on spectrophotometric measurement of NADPH formation rate, which is proportional to the G6PDH activity.

Protein concentration was measured in dilute liver homogenates according to Lowry et al. [7], using bovine serum as a standard. The enzyme activities were expressed as units of enzyme activity per miligram of protein.

The livers for reduced glutathione determination were homogenized in ice-cold 5% metaphosphoric acid, centrifuged at 3000g, 4°C for 10 minutes. The level of hepatic GSH was measured using BIOXYTECH GSH-400™ Assay kit produced by OXIS International Inc., Portland, USA. The method is based on chemical reaction proceeding in two steps. The first leads to the formation of thioethers between reagent and all mercaptans and the second leads to beta-elimination reaction which specifically transforms the thioethers obtained with GSH into a chromophoric thione, which has maximal absorbance wavelength at 400 nm.

The livers for hydrogen peroxide determination were prepared as for catalase determination, but buffer was replaced by 0.9% NaCl solution. The concentration was measured using BIOXYTECH H_2O_2-560™ Assay kit produced by OXIS International Inc., Portland, USA. The assay is based on the oxidation of ferrous ions (FeII) to ferric ions (FeIII) by hydrogen peroxide under acid conditions.

The liver concentration of MDA was determined as thiobarbituric acid reactive substances (TBARS) according to the method of Buege and Aust [8].

Data for the ten groups of animals were compared using one-way analysis of variance (ANOVA) followed by the Tukey-Kramer multiple comparison tests. The probability of $p<0.05$ was considered significant. The correlations were calculated between the groups.

Results

The results of this study demonstrated the changes in liver antioxidative parameters in rats after the administration of chlorfenvinphos at single doses.

The rat liver CAT activity increased statistically significantly in comparison to the control group, at the 1st hour after administration of chlorfenvinphos at lowest dose and at the 1st and 24th hour after administration of the insecticide in a dose of 0.1 and 0.5 LD_{50}. The activity observed after intoxication at the two lower doses of chlorfenvinphos was statistically significantly higher than that observed after intoxication with the insecticide at a dose of 0.5 LD_{50}, at the same time (1st hour). At the 48th hour after intoxication with chlorfenvinphos at a dose of 0.1 and 0.5 LD_{50}, CAT activity decreased. At this time, the CAT activity returned to the value observed in the control group (Tab. 1).

There was a statistically significant increase in GPx activity, compared to the control group, after intoxication with chlorfenvinphos at a dose of 0.02 and 0.5 LD_{50}, dur-

Table 1. Activity of CAT (U/mg protein) and GPx (U/g protein) in the liver of rats after acute intoxication with chlorfenvinphos (CVP).

	CAT	GPx
control	94.72±10.18 (n=15)	120.86±20.11 (n=10)
CVP-0.02LD$_{50}$		
1 h	163.47±27.95 ^{a} (n=8)	218.63±19.00 ^{a} (n=8)
24 h	96.64±23.66 ^{b} (n=8)	283.68±33.66 ^{ab} (n=8)
48 h	99.49±13.99 ^{b} (n=8)	189.13±24.51 ^{abc} (n=8)
CVP-0.1LD$_{50}$		
1 h	182.82±36.82 ^{acd} (n=6)	159.29±10.17 ^{abc} (n=8)
24 h	157.86±35.28 ^{acd} (n=6)	153.97±15.18 ^{abcd} (n=8)
48 h	96.82±16.30 ^{bef} (n=6)	169.57±21.34 ^{abcd} (n=8)
CVP-0.5LD$_{50}$		
1 h	133.48±19.09 ^{abceg} (n=7)	170.22±22.92 ^{abce} (n=8)
24 h	165.93±15.35 ^{acdfgh} (n=7)	186.38±19.65 ^{acef} (n=8)
48 h	104.43±15.11 ^{befhi} (n=6)	118.17±14.38 ^{bcdefghi} (n=8)

Legend: values expressed as means ± SD; n - the number of rats in the group; statistically significant in comparison with: a - control, b - 0.02LD$_{50}$ - 1h, c - 0.02LD$_{50}$ - 24h, d - 0.02LD$_{50}$ - 48h, e - 0.1LD$_{50}$ - 1h, f - 0.1LD$_{50}$ - 24h, g - 0.1LD$_{50}$ - 48h, h - 0.5LD$_{50}$ - 1h, i - 0.5LD$_{50}$ - 24h.

ing the whole experiment and at the 1st and 24th hour after treatment with 0.5 LD$_{50}$. The highest value of GPx was observed 24 hours after treatment with 0.02 LD$_{50}$ (Tab. 1).

The activity of GR increased in the group of rats treated with chlorfenvinphos at a dose of 0.1 LD$_{50}$ (3-5-fold) throughout the experiment, and at the first period, in the group of rats intoxicated with 0.02 LD$_{50}$ and 0.5 LD$_{50}$ (a twofold increase in activity compared to the control group, at the 24th hour).The increase in the activity of GR after treatment with chlorfenvinphos in a dose of 0.02 LD$_{50}$ was lower in comparison to the enhancement observed in the groups of rats intoxicated with the higher doses of the insecticide, at the same time (Tab. 2).

Treatment of rats with the lowest dose of chlorfenvinphos resulted in a decreased concentration of GSH in the liver of rats, at the 1st, 24th and 48th hours. Liver GSH concentration increased, statistically significantly compared to the control group, during the whole period of the experiment, after intoxication with chlorfenvinphos at a dose of 0.1 LD$_{50}$ and at the 1st and 24th hour after treatment with the highest dose of the insecticide (Tab 2).

There was no significant change in G6PDH activity in the liver of rats intoxicated with a dose of 0.02 LD$_{50}$, except for the 1st hour, when the activity of this enzyme decreased. The activity of G6PDH increased in comparison to the control at the 1st and 24th hour after treatment with chlorfenvinphos in a dose of 0.1 LD$_{50}$, and during the whole period after intoxication with the highest dose (Tab. 2). The highest values of this enzyme were observed at the

Table 2. Concentration of GSH (μmol/g tissue) and activity of GR and G6PDH (U/g protein) in the liver of rats after acute intoxication with chlorfenvinphos (CVP).

	GR	GSH	G6PDH
control	25.61±4.78 (n=8)	23.56±1.64 (n=7)	26.35±6.45 (n=7)
CVP-0.02LD$_{50}$			
1 h	37.98±4.12 ^{a} (n=7)	18.68±1.21 ^{a} (n=8)	16.27±2.55 ^{a} (n=8)
24 h	35.57±4.79 ^{a} (n=8)	17.87±1.34 ^{a} (n=9)	23.93±5.54 ^{b} (n=8)
48 h	28.18±4.42 ^{bc} (n=8)	17.55±1.21 ^{a} (n=8)	27.66±3.54 ^{b} (n=8)
CVP-0.1LD$_{50}$			
1 h	116.53±22.68 ^{abcd} (n=6)	70.15±5.08 ^{abcd} (n=7)	59.90±6.61 ^{abcd} (n=7)
24 h	100.56±17.43 ^{abcd} (n=6)	77.77±4.79 ^{abcde} (n=7)	68.42±9.46 ^{abcd} (n=7)
48 h	80.88±12.20 ^{abcde} (n=7)	72.18±2.57 ^{abcdef} (n=7)	30.18±5.32 ^{bef} (n=6)
CVP-0.5LD$_{50}$			
1 h	37.89±4.40 ^{aefg} (n=7)	35.12±3.22 ^{abcdefg} (n=6)	35.92±4.73 ^{abcef} (n=8)
24 h	62.68±17.56 ^{abcdefg} (n=7)	34.35±1.32 ^{acefgh} (n=8)	83.75±14.38 ^{abcdefh} (n=8)
48 h	27.14±6.11 ^{efgi} (n=9)	25.08±1.15 ^{bdefgi} (n=8)	63.87±10.26 ^{abcdgi} (n=7)

Explanations as in Table 1.

Table 3. Concentration of hydrogen peroxide (μmol/g tissue) and MDA (nmol/g tissue) in the liver of rats after acute intoxication with chlorfenvinphos (CVP).

	hydrogen peroxide	MDA
control	104.12±6.76 (n=10)	44.83±5.31 (n=12)
CVP-0.02LD_{50}		
1 h	149.92±10.50 ^{a} (n=9)	64.95±7.49 ^{a} (n=9)
24 h	123.42±12.10 ^{ab} (n=8)	57.64±5.43 ^{a} (n=8)
48 h	108.15±10.72 ^{bc} (n=8)	47.42±8.12 ^{b} (n=8)
CVP-0.1LD_{50}		
1 h	146.52±10.01 ^{acd} (n=8)	78.40±8.10 ^{acd} (n=7)
24 h	125.89±11.79 ^{abde} (n=8)	55.83±6.38 ^{ae} (n=8)
48 h	110.65±12.73 ^{bcef} (n=8)	48.24±8.14 ^{be} (n=8)
CVP-0.5LD_{50}		
1 h	127.01±5.90 ^{abdeg} (n=8)	47.63±9.78 ^{be} (n=8)
24 h	125.02±10.92 ^{abdeg} (n=8)	26.76±1.20 ^{abcdefgh} (n=7)
48 h	153.10±6.98 ^{acdfghi} (n=8)	67.46±3.72 ^{adeghi} (n=9)

Explanations as in Table 1.

1st and 24th hours after intoxication with chlorfenvinphos at the dose of 0.1 LD_{50}, and at the 24th and 48th hour after intoxication with insecticide at the dose of 0.5 LD_{50}.

The concentration of hydrogen peroxide increased statistically significantly compared to the control group at the 1st and 24th hours after intoxication with chlorfenvinphos in a doses of 0.02 and 0.1 LD_{50}, and during the examined period after intoxication with insecticide at a dose of 0.5 LD_{50} (Tab. 3).

The results of this study demonstrated a rapid increase in liver MDA levels at the 1st and 24th hours after treatment (almost twofold higher concentration than in the control group) at the lower doses of chlorfenvinphos. The MDA level returned to the control value at the 48th hour (Tab. 3). After treatment of rats with the highest dose of chlorfenvinphos the MDA concentration decreased at the 24th hour and increased at the 48th hour.

Analysis of the results demonstrated a correlation between the activities of some antioxidative enzymes and GSH concentration. A positive correlation (r=0.7619, p=0.0001) was found between GR activity and GSH concentration and a negative correlation (r= -0.4695, p=0.0001) between the activity of GPx and GSH concentration. A negative correlation (r= -0.4318, p=0.0001) was also found between the activities of G6PDH and GPx. There was a positive correlation (r= 0.3541, p=0.004) between CAT activity and hydrogen peroxide concentration, although we found a slightly negative correlation (r= -0.2515, p=0.047) between GPx activity and hydrogen peroxide concentration. The concentration of hydrogen peroxide and concentration of GSH were correlated (r=0.5651, p=0.0001) as well. A negative correlation (r= -0.3279, p=0.006) was observed between the activity of G6PDH and MDA concentration.

Discussion

In the present study, the activities of CAT and glutathione-dependent enzymes, GSH level as well as the concentration of hydrogen peroxide and MDA were determined in the liver of rats acutely intoxicated with chlorfenvinphos.

The primary antioxidant enzymes in mammalian tissues are superoxide dismutase (SOD), CAT and GPx. This work shows the increased activity of CAT at the first hour after intoxication with chlorfenvinphos at the dose of 0.02 LD_{50} and at 1st and 24th hours at a dose of 0.1 and 0.5 LD_{50}. Although the activity of GPx was significantly higher in comparison to control at the 1st and 24th hour at the highest dose and during the whole examined period following treatment with the two lower doses. It is interesting to note that GPx reached the highest value at the 24th hour after intoxication with the insecticide at a dose of 0.02 LD_{50}. Thus, the changes in the activity of the enzymes were not dose-dependent.

There is little data concerning the influence of pesticide on the activity of antioxidant enzymes and for this reason it is very difficult to compare our results with those reported by other authors. In the mice receiving diquat at different doses - bispyridyl herbicide acting as prooxidant, the activity of hepatic GPx tended to decrease when herbicide dose increased. Other investigators reported different changes in GPx activity after the rodents received prooxidative agents [9, 10]. Tanguchi et al. [11] observed a decreased activity of liver GPx after treatment of rats with N-nitrosodimethyloamine in a single dose of 30 mg/kg b.w. However, after treatment of rats with both higher and lower doses of this compound, the activity of GPx did not exhibit any changes.

We also found the elevated levels of H_2O_2 at 1 and 24 hours in the liver of rats intoxicated with chlorfenvinphos at a dose of 0.02 and 0.1 LD_{50}, while the highest level was observed at hour 1. The increased level of H_2O_2 was also observed after treatment of rats with chlorfenvinphos at a dose of 0.5 LD_{50}, but the highest value was observed at 48 hours after intoxication. The high concentration of H_2O_2 was accompanied by high activity of GPx and CAT. CAT and GPx play a significant role in the elimination of hydrogen peroxide. Catalase is the most efficient enzyme known. It is so efficient that it cannot be saturated by H_2O_2 at any concentration [12]. Because of differencies between catalase and peroxidase in the Michaelis constant to H_2O_2, their contribution to hydrogen peroxide detoxification is also different. It has been suggested that GPx is responsible for the detoxification of H_2O_2, when it is present in low concentration, whereas CAT plays its role when GPx pathway reaches saturation with substrate

[12, 14, 15]. Results of the present work indicate a temporary increase in the activity of GPx after intoxication of rats with highest dose of chlorfenvinphos and during the whole period after treatment with the lower doses. Interpretation of the results is difficult. In acute intoxication with the lowest dose of chlorfenvinphos in the first period after treatment, the activity of two enzymes responsible for H_2O_2 detoxification increased. However, because the activity of GPx is sufficient, CAT activity returned to control value. It seems that the changes in the level of H_2O_2 confirmed the above-mentioned assumption. In other words, H_2O_2 concentration returns to control value at the 48th hour of intoxication. Treatment with chlorfenvinphos at a dose of 0.1 LD_{50} could result in a rapid and early increase in the superoxide anion level, which suggests a simultaneous increase in SOD activity as we have previously reported [16]. The increased activity of SOD was accompanied by the increase in the activity of CAT, at the 1st and 24th hour of intoxication. Laszlo et al. [17] and Kono et al. [18] have reported that superoxide anion inhibits CAT action. However, we can assume that the increase in the activity of SOD is not sufficient to detoxify the excess of superoxide anion. This could lead to a decrease of CAT activity, which was observed in this experiment, at the 48th hour.

There are statistically significant correlations between the liver H_2O_2 level and the activity of GPx (negative) and between H_2O_2 level and CAT activity (positive). These correlations seem to confirm earlier suggestions. The increase in hepatic CAT activity can be explained as the response of liver to high levels of H_2O_2.

In the present work, the concentration of GSH, a key cellular non-enzymatic antioxidant, was also determined. Numerous enzymes participate in glutathione metabolism. GPx catalyses the formation of oxidized glutathione (GSSG) during the reduction in hydroperoxides [19, 20]. GSH can be regenerated from GSSG by glutathione reductase [20]. In this study, the increase in the concentration of liver GSH was found at the 1st and 24th hours after treatment of rats with chlorfenvinphos at a dose of 0.5 LD_{50} and during the whole examined period after treatment with the insecticide at a dose of 0.1 LD_{50}. The highest value was noted at the 24th hour after intoxication. However, after treatment of rats with chlorfenvinphos at the lowest dose, a decrease in GSH level was observed.

In the present study, liver GSH concentration and GPx activity were negatively correlated, for the lower dose but positively for two higher. As it is known, this enzyme is involved together with GSH in the protection of organism against reactive oxygen species. GSH has many important functions in the cell. It directly participates in the scavenging of free radicals: hydrogen peroxide, superoxide anion and hydroxyl radicals. GPx catalyses the formation of GSSG from GSH during the reduction of free radicals [20]. Based on the above, the negative correlation between GSH and GPx seems to be clear. Other authors have reported that the exposure of organism to the generated oxidative stress substances results in the enhanced level of GSH [11, 21, 22]. This increase is caused by the enhancement of its synthesis after induction of γ-glutamylcysteine synthetase and this mechanism is considered to be adaptative [11]. This may explain the increased concentration of GSH in acute intoxication of rats with chlorfenvinphos. However, after intoxication with the lowest dose of insecticide a decreased level of GSH has been observed. Variations in GSH level during oxidative stress may result from modification in synthesis and/or loss. The mechanisms responsible for maintainance of GSH homeostasis in different tissues are poorly documented, especially in pathological conditions. However, the significance of the glutathione redox cycle in the protection against oxidative stress is well known [12, 23]. Depleted glutathione levels after intoxication with chlorfenvinphos at the lowest dose may be caused by its involvement in scavenging of H_2O_2. This reaction is catalysed by GPx, as mentioned above [22]. Decreased GSH concentration was accompanied by a significant increase in GPx activity. As a result of this reaction, GSH is oxidized and therefore the reduced glutathione level is diminished.

The complicated reactions between the antioxidant enzymes make interpretation of the results difficult. Recently, Spolarics et al. [12] reported a depleted GSH content by 75% in Kupffer cells after CAT inhibition in the presence of 0.2 mM H_2O_2. However, in acute intoxication with chlorfenvinphos, at the time when GSH concentration decreased, CAT activity neither changed nor increased. In acute intoxication with chlorfenvinphos, we assume that H_2O_2 is detoxified mainly by GPx.

Zhang et al. [24] observed that increase in cAMP level causes phosphorylation and inhibition of G6PDH activity. The others reported that some of the muscarinic receptors mediate their effects by inhibiting adenylate cyclase, thereby decreasing cellular cAMP level [25]. Moreover, the signs and symptoms of intoxication with organophosphate insecticides are due to activation of cholinergic muscarinic and nicotinic receptors. Thus the activation of muscarinic receptors seems to influence activity of G6PDH.

The GSH-dependent antioxidative system consists of two enzymes: GPx and GR [26]. GR catalyses the reduction of GSSG to GSH. By the contrast to GPx, this enzyme is involved in the maintainance of glutathione in reduced form and owing to this, GSH plays its antioxidant functions [26, 27].

In acute intoxication with chlorfenvinphos, the activity of GR increased at the 1st and 24th hour after treatment of rats with the highest and the lowest doses of the insecticide, and during the whole examined period after treatment at a dose of 0.1 LD_{50}. The high positive correlation between GR activity and GSH level suggests that the increase in reductase activity is the defence mechanism by which GSH availability is preserved.

In maintaining the intracellular pool of GSH, NADPH - the cell principal reductant, plays a critical role. The pentose phosphate pathway is considered to be a major source of cellular reducing power [28, 29]. G6PDH is the

key enzyme of this pathway [24, 29]. NADPH is required for the functions of GPx (via glutathione) as well as for stability of CAT [12]. Cell death induced by oxidative stress is dependent on the maintainance of proper activity of G6PDH [24, 29]. In view of the above data we suppose that increased activity of G6PDH after intoxication with chlorfenvinphos is a defence mechanism against the insecticide-induced oxidative stress. In the intoxication with chlorfenvinphos at the lowest dose, a decrease in the activity of G6PDH was observed at the 1st hour after treatment and then the activity of enzyme returned to the control value. This phenomenon can explain the decreased level of GSH, which has been observed in these groups of rats. The activity of G6PDH and concentration of GSH were positively correlated. This correlation is clear in view of the literature cited above.

The estimation of antioxidative enzymes and non-enzymatic antioxidative parameters after intoxication with chlorfenvinphos has shown that the defence reaction of the organism against toxic influence of the insecticide is sometimes greater after intoxication with the lower dose. This was evident in the estimation of GPx activity, which showed a more significant increase after intoxication with a dose of 0.02 LD_{50}. The others reported that mammalian tissues react on the small or medium oxidative stress by the overproduction of antioxidants, like SOD, CAT, GPx or GSH [30]. In the intoxication with chlorfenvinphos at the dose of 0.1 LD_{50}, this phenomenon probably takes place.

Oxidative stress affects cellular integrity only when antioxidative are no longer capable of coping with ROS. ROS reacts with the unsaturated fatty acid of cellular or subcellular membranes. Therefore, they lead to peroxidation of membrane lipids. The oxidative stress caused by different xenobiotics is often estimated by the level of MDA [11, 31, 32, 33]. The results obtained in this study show the enhancement level of MDA at the 48th hour after intoxication with chlorfenvinphos at a dose of 0.5 LD_{50} and as early as at 1 hour after intoxication with two lower doses of insecticide. At the 24th hour of intoxication with chlorfenvinphos of 0.5 LD_{50}, a decrease of MDA level was observed. This indicated that the prooxidant effect of the highest dose of chlorfenviphos becomes significant only after the 48th hour of intoxication, while the same effect of lower doses is evident much earlier. Thus, in the present study, enhancement of lipid peroxidation was not dose-dependent, but was rather related to the time after treatment. The increased level of MDA in the intoxication with organophosphate insecticide has been reported by other authors. A dose-dependent increase in its concentration was found in the renal tubules of rats after treatment (in vitro) with acephate [32, 33]. The MDA concentration increased also in the skeletal muscles of rats intoxicated with diisopropylfluorphosphate [31].

The correlations between MDA level and examined elements of enzymatic and non-enzymatic antioxidative system were determined. A statistically significant correlation was found only between MDA level and G6PDH activity. This correlation was negative. When G6PDH activity reached the maximum value, the MDA concentration diminished.

In conclusion, it is suggested that G6PDH play a key role in the defence against oxidative stress induced by treatment with chlorfenvinphos.

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