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Original Research

Assessment of Lipid Peroxidation in Rat Tissues in Subacute Chlorfenvinphos Administration

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Abstract

Objective: To study the levels of malonyldialdehyde, marked as the concentration of substances reacting with thiobarbituric acid in liver, brain, serum and kidney in subacute intoxication with low doses of chlorfenvinphos, an organophosphate insecticide.

Materials and Methods: The study used male Wistar rats, body weight 250 – 280g. The animals received intragastrically, by a gastric tube, once daily 0.1ml/100g of olive oil (the control group) or oil solution of chlorfenvinphos at a dose of $0.02LD_{50}$ (0.3 mg/kg b.w.) (the experimental groups). After 14 and 28 days of the experiment, blood samples were collected by cardiac puncture to obtain serum; liver, kidney and brain sections were taken from the animals in anaesthesia. The level of malonyldialdehyde (as TBARS) was determined in homogenates of the organs and in serum. Additionally, serum cholinesterase activity was determined.

Results: There was an increase in the tissues as well in the serum malonyldialdehyde level. Proportionally the highest increase in TBARS, as compared to the control, was observed in the liver on day 28 of chlorfenvinphos administration and in the kidney on day 14 of the exposure.

Conclusion: In subacute chlorfenvinphos intoxication, lipid peroxidation is increased in the rat liver, serum, kidney and brain, which may cause various health effects in the population exposed to its action.

Keywords: subacute chlorfenvinphos administration, lipid peroxidation.

Introduction

The mechanism of the toxic effect of organophosphate compounds involves the inhibition of acetylcholinesterase and other non-specific esterases through phosphorylation at –OH serine in the esterase centre of the enzyme. This mechanism is the same for all insecticides of the group, irrespective of differences in their chemical structure [1]. The inhibition of the activity of cholinesterase enzymes causes an increase in the level of endogenous acetylcholine in the organism and results in its binding to muscarinic and nicotinic receptors in both the peripheral and central nervous systems. This increase in the CNS disturbs the balance between neurotransmitters and causes the onset of acute intoxication symptoms [1].

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The symptoms of acute intoxication with organophosphates have been well described, while the effects of chronic exposure to these compounds are not completely clear. Many authors postulate that they may have an effect on redox processes in a number of organs, thus leading to disturbances in these processes and causing enhancement of lipid peroxidation, both in acute and chronic intoxication by these compounds [2-5].

As increased generation of reactive oxygen species and lipid peroxidation induced by these species underlies many diseases, it is extremely important to determine the effect of organophosphate insecticides on lipid peroxidation processes [6-8].

Therefore, the study objective was to assess the levels of malonyldialdehyde, marked as the concentration of substances reacting with thiobarbituric acid, in liver,

brain, serum and kidney in subacute intoxication with low doses of chlorfenvinphos.

Material and Methods

The study used male Wistar rats, body weight 250 – 280g, which were kept in metal cages and had free access to drinking water and standard granulated diet. The animals received intragastrically, by a gastric tube, once daily 0.1ml/100g of olive oil (the control group) or oil solution of chlorfenvinphos, i.e. 2-chloro-1-(2.4-dichlorophenyl) vinyldiethyl phosphate (CVP) at a dose of $0.02LD_{50}$ (0.3 mg/kg b.w.) (the experimental groups). The LD_{50} for chlorfenvinphos was 15 mg/kg b.w.

The study was approved by the Ethics Committee at the Medical University of Białystok.

Collection of Samples

After 14 and 28 days of the experiment, blood samples were collected by cardiac puncture to obtain serum. Liver, kidney and brain sections were taken from the animals in vetbutal anaesthesia.

Lipid Peroxidation Estimation

The level of malonyldialdehyde, as a substance that reacts with thiobarbituric acid (TBARS), was determined in homogenates of the organs and in serum according to the method of Buege [9]. The 10% homogenates of tissues in 0.15M KCl were centrifuged at 10000xg for 30 min. To 0.5 ml of supernatant or 0.5 ml of serum 0.5 ml of 50% trichloroacetic acid were added and centrifuged again, 5000xg, 5 min. After the final centrifugation, the tubes with 0.5 ml of supernatant and 0.5 ml of thiobarbituric acid covered with aluminium foil were kept in a water bath at 90°C for 1 hour. The absorbance was read at 540 nm at room temperature against the blank and then concentration of thiobarbituric acid reactive substances was read from standard calibration curve, which was plotted using 1, 1, 3, 3' tetra – ethoxy propane. The resulting concentration of TBARS were presented in micromoles of TBARS per dm^3 of serum or in nanomoles of TBARS on g of tissue.

Cholinesterase Estimation

Additionally, serum cholinesterase (ChE) activity, as the index of exposure to organophosphate insecticides, was determined spectrophotometrically, according to the Ellman method [10].

The data were subjected to statistical analysis. The results obtained in the respective groups were referred to the control and correlations were investigated between them. Non-parameteric U Mann-Whitney test, being an alternative non-parametric form of t-Student's test for variables of similar interpretation, was used. According to Bolferoni's inequality, differences between the groups were statistically significant for $p < 0.05$.

Pearson's correlation coefficients were determined, with statistical significance at $p < 0.05$.

Results

A decrease in rat serum ChE activity was found in subacute chlorfenvinphos intoxication on day 28. At the same time, a statistically significant increase was observed in serum TBARS level (Table 1). However, a rise in lipid peroxidation index was found earlier, i.e. after 14 days of exposure. TBARS concentration in the liver of rats receiv-

Table 1. Serum ChE activity and levels of substances reacting with thiobarbituric acid in serum and chosen rat tissues in subacute intoxication with chlorfenvinphos.

Parameter	Period of CVP administration			
	14 days		28 days	
	0	0.02 LD_{50}	0	0.02 LD_{50}
Serum ChE activity (U/dm^3)	1428.22±189.58 n=6	1496.23±157.41 n=7	1416.01±164.76 n=7	1226.80±150.36[a] n=7
Serum TBARS level ($\mu mol/ dm^3$)	2.83±0.34 n=7	3.28±0.62 n=8	3.25±0.47 n=7	6.02±0.63[ab] n=8
Liver TBARS level (nmol/g tissue)	45.89±7.29 n=7	62.37±12.43[a] n=7	40.67±9.13[b] n=8	109.68±21.36[ab] n=6
Kidney TBARS level (nmol/g tissue)	19.18±3.23 n=6	46.16±9.13[a] n=7	20.23±1.61 n=8	36.13±1.88[ab] n=10
Brain TBARS level (nmol/g tissue)	23.98±2.46 n=7	44.32±8.93[a] n=7	21.28±2.57 n=10	40.57±3.14[a] n=8

The Table contains: mean ± standard deviation; n – number of rats in a group; a – statistically significant compared to the control; b – statistically significant compared to the group receiving CVP for 14 days

ing chlorfenvinphos increased with the time of exposure. On day 14, it was 136% of the control value, reaching the level of 270% on day 28 (Table 1). In the kidneys, TBARS concentration decreased statistically significantly with time of exposure, while in the brain it remained at a constant elevated level (Table 1). After 14 days of intoxication, the level of TBARS increased by 160% for the kidney and by 85% for the brain, as compared to the respective control. On day 28, TBARS concentration in the kidney was approximately 89% higher than in the control group, its level in the brain being unchangeably elevated.

Proportionally the highest increase in TBARS, as compared to the control, was observed in the liver on day 28 of chlorfenvinphos administration and in the kidney on day 14 of the exposure.

The changes in TBARS in the liver correlated positively with those observed in the serum ($r = 0.7443$, $p = 0.0001$). The correlation was weaker, yet statistically significant, between its levels in the liver and the kidneys ($r = 0.5245$, $p = 0.007$), and between the brain and the liver ($r = 0.5478$, $p = 0.005$).

Discussion

Chlorfenvinphos – an organophosphate insecticide, posing a risk to those professionally involved in its production and use in agriculture, was chosen for the current study. The general population may be exposed to this compound by the consumption of polluted food products [11]. Chlorfenvinphos occurs in trace amounts in pharmaceutical products with lanolin, a wool-fat obtained from sheep, as chlorfenvinphos is used to combat parasites in these animals [12].

A study on acute poisoning with chlorfenvinphos has revealed that apart from its major toxic mechanism, i.e. inhibition of cholinesterase activity, this compound also causes some changes in the activity of antioxidative enzymes – superoxide dismutase and catalase – both in liver and in erythrocytes, as well as lipid peroxidation increase [13]. Studies on other organophosphate insecticides administered at multiple doses have also showed intensification of malonyldialdehyde formation (lipid peroxidation index) and enhancement of changes in the activity of antioxidative enzymes in many tissues and organs [2, 4].

Cellular membranes contain polyunsaturated fatty acids susceptible to the action of free oxygen radicals that initiate membrane lipid peroxidation, thus leading to disturbances in the structure and function of cells [14, 15]. Lipid aldehydes generated during breakdown of lipid superoxides are especially dangerous for the organism. These aldehydes, although less reactive than superoxides, can easily migrate at a considerable distance and have a longer (a few minute) half-life. Therefore, lipid aldehydes can react with other molecules far away from the site of their origin [14, 16].

The increased formation of reactive oxygen species and the cell dysfunction they induce have been observed in many diseases, such as arrhythmia, hypertension, damage to skeletal muscles, neuronal damage in Parkinson's disease, diabetes, and Alzheimer's disease [6, 7, 8]. Thus, any environmental factor affecting lipid peroxidation poses a real risk to the health of the population exposed to its action.

The current study demonstrates that in subacute intoxication with low doses of chlorfenvinphos that best resemble those of environmental exposure, lipid peroxidation occurs and it becomes enhanced before serum cholinesterase activity, i.e. the index assessed in professional OP exposure is reduced. In our earlier study we observed no change of serum ChE activity at 24 h of acutely intoxicated rats with a dose of 0.02 LD_{50} [17].

The increase in liver TBARS level observed in the current study is not surprising. According to other researchers, organophosphate insecticides, including chlorfenvinphos, are metabolized in the liver in the presence of cytochrome P450-dependent mixed function monooxygenases, which hydroxylate organophosphates to hydrophilic intermediary products [18]. These intermediary products are conjugated with endogenous compounds, mainly with glucoronic acid, and excreted with urine [18].

Animal studies have revealed that a single dose of chlorfenvinphos reduces toxicity of the subsequent dose administered within a 24h period and that its metabolic rate is increased [19, 20, 21]. A single oral dose of chlorfenvinphos causes a rise in cytochrome P450 level and in the activity of cytochrome P450 reductase by 30%, in cytochrome b5 concentration by 21%, and an increase in the activity of enzymes related to cytochrome P450, including aminopyrin demetylase (by 40%) and aniline hydroxylase (by 27%) in the microsomal fraction of the liver. Similar changes in the above parameters were observed after administration of phenobarbital, a known cytochrome P450 inductor, to experimental animals, 24 hours before chlorfenvinphos was applied. Chlorfenvinphos, by causing a rise in the concentration of cytochrome P450 and the related enzymes, may lead to the increased production of reactive oxygen species [21]. In chronic intoxication, when chlorfenvinphos (1.000 ppm) was added to the rats' fodder for two months, the induction of mixed function monoxygenases was observed in microsomes. This causes acceleration of phase I reaction, which shortens the time of the toxic action of chlorfenvinphos on acetylocholinesterase and speeds up detoxification through faster binding of its metabolites to glucuronic acid [18].

However, the increase in lipid peroxidation observed in the liver seems to be caused by chlorfenvinphos metabolism in this organ, while its induction and a further rise in the generation of reactive oxygen species leads to enhancement of this process along with exposure duration.

A high positive correlation is found to occur between lipid peroxidation process in liver and serum. As shown by literature data, increased peroxidation of lipids in many tissues and organs leads to their 'leakage' to the blood circulation and shift to other organs and tissues [8]. The current study results seem to suggest that the elevation of serum TBARS level in subacute poisoning with chlorfenvinphosis is mainly due to the rise in the liver. However,

literature data vary and for example, a 28-day malathione administration caused a decrease in TBARS in serum and a simultaneous increase in brain [4].

We found a very high level of lipid peroxidation index in the kidney on day 14 of chlorfenvinphos administration and its reduction on day 28, as compared to the values observed in the early phase of intoxication, thus indicating enhancement of adaptive processes in this organ and elevated activity of antioxidative enzymes. Enhancement of lipid peroxidation processes in the kidneys have also been reported by other authors studying both acute and chronic exposure to organophosphate compounds [2, 3, 22]. The oxidative stress in the kidney is most likely caused by the effect of oxygen metabolites, the finding reported from intoxication with bidrin – another organophosphate compound [22]. The positive correlation observed in the current study between TBARS levels in the liver and kidney seems to confirm this fact.

Brain tissue is particularly susceptible to oxidative damage, as it is rich in polyunsaturated fatty acids which easily undergo peroxidation. Moreover, the brain uses a relatively large amount of oxygen at a rather low activity of antioxidative enzymes as was reported by other authors [14, 15].

We observed an increase in the level of TBARS already on day 14 of exposure and the concentration remained at this elevated level up to day 28. At the same time, there was a high positive correlation found between the level of TBARS in liver and brain, suggesting that the increase in the generation of reactive oxygen species in liver due to metabolic processes of chlorfenvinphos contributes to the enhancement of lipid peroxidation and thus to the rise in brain TBARS concentration. We suppose that as reported by other authors [15], low activity of antioxidative enzymes in this organ results in stabilization of their level throughout the study period.

Concluding, in subacute chlorfenvinphos intoxication, lipid peroxidation is increased in the rat liver, serum, kidney and brain, which may cause various health effects in the population exposed to its action.

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