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Original Research

A Low Dose of Chlorfenvinphos Affects Hepatic Enzymes in Serum and Antioxidant Enzymes in Erythrocytes and Liver of Rats

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Abstract

The aim of this study was to investigate the effect of chlorfenvinphos administered at a single dose of 0.02 LD_{50} on the activity of serum of liver damage indicatory enzymes, such as β-glucoronidase (BGR), acid phosphatase (AcP) and alanine and aspartate aminotransferases (ALT and AST), as well as on the activities of superoxide dismutase (SOD) and catalase (CAT) in erythrocytes and liver.

The animals were divided into two groups: the control group, which received oil, and the experimental groups, which received oil solution of chlorfenvinphos at a dose of 0.02 LD_{50}. After 1, 24 and 48 hours of intoxication with chlorfenvinphos the blood samples were collected and livers were quickly removed.

This study indicates that acute intoxication with chlorfenvinphos administered at a dose of 0.02 LD_{50} leads to liver function disturbances, which is a likely result of increased generation of reactive oxygen species.

Keywords: chlorfenvinphos, low dose, liver enzymes, SOD, CAT

Introduction

Organophoshate compounds, including chlorfenvinphos (widely used in Poland), are detectible in food as well as in the environment and they endanger many people.

Symptoms of intoxication with organophosphate insecticides are due to the inhibition of the activity of cholinesterases, leading to acetylcholine accumulation. The accumulation rate depends on the degree of AChE activity inhibition [1, 2]. Moreover, these compounds lead to liver function disorders, as shown by other authors and in our own studies [3-7]. The mechanism causing changes in the activity of liver parameters has not been fully elucidated [6, 8]. Literature data as well as our own experiments suggest that this damage may result from the production of reactive oxygen species (ROS) [8-12]. Both the increased production of reactive oxygen species and attenuation of the antioxidant barrier of the organism are likely to induce oxidative stress, thus leading to organ damage [8, 10, 12]. There are only a few reports on the changes in the activity of antioxidant enzymes after intoxication with organophosphate compounds. An increase in the activity of CAT and other antioxidant parameters was reported by authors investigating fish exposed to dichlorvos [12]. We have observed changes in the activity of catalase and superoxide dismutase, both in erythrocytes and in the liver after chlorfenvinphos administration. However, higher doses of the compound (0.1 and 0.5 LD_{50}) were used in our studies [10, 11].

Therefore, the aim of the present study was to investigate the effect of chlorfenvinphos administered at a single dose of 0.02 LD_{50} on the activity of serum liver damage indicatory enzymes, such as β-glucoronidase (BGR), acid phosphatase (AcP) and alanine and aspartate aminotransferases (ALT and AST), as well as on the activity of superoxide dismutase (SOD) and catalase (CAT) in erythrocytes and liver.

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Table 1. Activity of the serum BGR, AcP, ALT and AST (U/dm^3) after acute intoxication with chlorfenvinphos at low dose (CVP).

Group	BGR	AcP	ALT	AST
control	8.91±1.03 (n=12)	95.87±7.16 (n=12)	19.44±2.76 (n=12)	39.60±6.96 (n=11)
CVP - 0.02 LD_{50}				
1 h	12.32±1.41[a] (n=8)	100.18±17.31 (n=8)	27.40±1.56 [a] (n=8)	39.49±7.56 (n=8)
24 h	9.94±1.02 [b] (n=8)	105.21±11.14 (n=8)	26.12±1.21 [a] (n=8)	34.92±6.12 (n=8)
48 h	8.11±0.85 [bc] (n=8)	97.76±14.10 (n=8)	20.64±3.72 (n=8)	41.04±3.72 (n=8)

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.

Materials and Methods

The studies were conducted on male Wistar rats of 200±20 g 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) at a dose of 0.02LD_{50} (LD_{50}=15 mg/kg b.w.). After 1, 24 and 48 hours of intoxication with chlorfenvinphos the blood samples were collected and livers were quickly removed. The livers were placed in iced 0.9% NaCl containing 0.16 mg/ml heparin. Our study was approved by the Local Ethical Committee.

Serum activity of BGR was determined using the Bergmayer method [13]. Determination of AcP, ALT and AST activity was performed using assay kits produced by LaChema, Czech Republic. CAT activity was measured in the liver and erythrocyte as described by Aebi [14]. Determination of liver as well as erythrocyte activity of SOD was performed using BIOXYTECH SOD-525^{TM} Assay kit produced by OXIS International, Inc., Portland, USA. The liver concentration of protein was determined according to the method of Lowry [15].

Data for all 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.

Results

An increase was observed in serum BGR activity at the 1st hour after chlorfenvinphos administration at the dose tested. No statistically significant change was noted in the activity of AcP, compared to the control group. The activity of alanine aminotransferase increased statistically significantly, compared to control, at the 1st and 24th hour of intoxication, while the activity of aspartate aminotransferase remained unchanged (Table 1).

SOD activity, both in erythrocytes and in the liver, increased statistically significantly, compared to control, at the 1st hour after chlorfenvinphos administration. It returned to control values at the 24th hour in erythrocytes, but was reduced statistically significantly in the liver, compared to control. An increase in CAT activity in erythrocytes was noted at the 1st and 24th hour of intoxication, while in the liver only at the 1st hour after administration of chlorfenvinphos (Table 2).

Discussion

Determinations of the activities of serum indicatory enzymes, such as BGR, AcP, ALT and AST were used to evaluate liver function in acute intoxication with chlorfenvinphos at a dose of 0.02 LD_{50}.

Increased serum BGR activity in intoxication with organophosphate compounds has been observed by many authors [16, 17, 18]. We have noted an increase in serum BGR activity in a study on the intoxication with high doses of chlorfenvinphos (0.5 and 0.1 LD_{50}) [3]. The increase was, however, much more pronounced and remained so for a longer period after intoxication. Enhancement of BGR activity is a typical symptom of intoxication with organophosphate insecticides and, according to some authors, this enzyme may be a sensitive indicator of intoxication with insecticides of that group [19]. Many authors have demonstrated that the increase in serum BGR activity correlates with a reduced activity in lysosomes and microsomes of the liver [17, 18, 20]. Thus, an increase in serum BGR, as well as AcP activity, accompanied by a reduction in the activity in the liver, may indicate an increase in permeability of cellular and subcellular membranes of hepatocytes. This phenomenon was observed for higher doses of chlorfenvinphos in earlier studies [3, 4]. The hepatotoxic action of xenobiotics is also manifested in the increased activity of aminotransferases ALT and AST in serum. Various organophosphate insecticides demonstrate this kind of action [21, 22]. Differences in the behaviour of these two aminotransferases, which have been observed in this paper, may result from their subcel-

Table 2. Activity of SOD and CAT in the erythrocyte (U/cm^3) and liver (U/mg protein) of rats after acute intoxication with chlorfenvinphos at low doses.

Group	SOD - erythrocyte	SOD - liver	CAT - erythrocyte	CAT - liver
control	226.29±16.11 (n=12)	268.73±24.59 (n=9)	110.11±8.17 (n=14)	94.72±10.18 (n=15)
CVP - 0.02 LD_{50}				
1 h	323.96±28.84 [a] (n=8)	360.79±27.68 [a] (n=8)	129.62±8.73 [a] (n=10)	163.47±27.95 [a] (n=8)
24 h	235.79±14.38 [b] (n=8)	220.15± 16.32 [ab] (n=6)	129.45±10.11 [a] (n=7)	96.64±23.66 [b] (n=8)
48 h	238.51±21.02 [b] (n=9)	251.72± 19.47 [b] (n=8)	108.22±8.38 [bc] (n=7)	99.49±13.99 [b] (n=8)

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.

lular location. Alanine aminotransferase, located mainly in hepatocyte cytoplasm, is released to the blood already when damage to cytoplasmic membranes occurs [23, 24]. Aspartate aminotransferase is situated mainly in mitochondria and thus its enhanced activity in the blood may indicate disorders in the functions of mitochondrial membranes and their increased permeability [23]. According to many authors, however, the increase in the activity of aminotransferases in serum does not always correlate with liver damage degree [25, 26]. Aminotransferases have diverse organ specificity. Alanine aminotransferase is highly liver-specific, while aspartate aminotranferase is also found in the heart, the brain, the kidneys and in skeletal muscles, which makes it less organ-specific [25, 26].

It has been demonstrated previously that two mechanisms, namely hypoxia and overproduction of reactive oxygen species, are responsible for liver damage in intoxication with chlorfenvinphos [11, 27]. In the present study, changes in SOD activity were found both in erythrocytes and in the liver. The increased activity of this enzyme observed at the first hour of intoxication in erythrocytes and in the liver, is undoubtfully a defense reaction of the organism to the increasing concentration of superoxide anion radical already present at that time. The increase in SOD activity was accompanied by increased activity of the other enzyme CAT preventing an increase in the concentration of reactive oxygen species. A simultaneous increase in SOD and CAT activity, observed in erythrocytes and in the liver, is an advantageous phenomenon. SOD is an ”incomplete antioxidant”, which by scavenging superoxide anion radical contributes to overproduction of hydrogen peroxide [28]. Thus, the simultaneous increase in CAT activity prevents excessive generation of this reactive oxygen form [29]. Catalase is the major enzyme responsible for hydrogen peroxide removal in normal erythrocytes. Breaking down hydrogen peroxide, this enzyme does not cause generation of any other reactive oxygen species [30]. However, decreased liver SOD activity at the 24th hour of intoxication may result from overproduction of hydrogen peroxide, as SOD is inactivated by the products of its own reaction. At the same time, as suggested by our earlier studies, concentration of hydrogen peroxide in the liver increases [31].

The present study indicates that acute intoxication with chlorfenvinphos administered in as small a dose as 0.02 LD_{50} leads to liver function disturbances, which is a likely result of increased generation of reactive oxygen species.

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