Polish Journal of Environmental Studies Vol. 13, No. 1 (2004), 69-72

The Influence of Pretreatment with N-Acetylcysteine on Serum Cholinesterase Activity and Liver Glutathione Levels in Rats Intoxicated with Chlorfenvinphos

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Received: 10 April, 2003
Accepted: 2 June, 2003

Abstract

This study was intendent to examine if N-acetylcysteine (NAC) changes liver GSH levels and influences ChE serum activity in rats intoxicated with chlorfenvinphos. The studies were conducted on male Wistar rats of 200±20 g body weight. One group of rats was pretreated with 0.1% water solution of NAC. NAC was administered in drinking water 24h before intoxication. The control groups received oil intragastrically by stomach tube in the amount of 0.1ml/100g (I), immediately or after NAC pretreatment. The experimental groups received oil solution of chlorfenvinphos in a dose of 0.02 LD_{50} or 0.1 LD_{50} immediately or after pretreatment with NAC. One and 24 hours after intoxication with chlorfenvinphos (or after NAC pretreatment) the blood samples were collected and livers were quickly removed and placed in iced 0.9% NaCl containing 0.16 mg/ml heparin. ChE serum activity and GSH level were measured.

The results of this study demonstrated the changes in serum ChE activity and liver glutathione levels in the rats after administration of chlorfenvinphos at single doses. The results reported here indicate that NAC influences a decreased level of GSH in the liver of chlorfenvinphos-intoxicated rats and does not prevent ChE inhibition.

Keywords: chlorfenvinphos, NAC, ChE, GSH

Introduction

Chlorfenvinphos is an organophosphate insecticide widely used alone or in combination in Poland. The signs and symptoms of intoxication with organophosphate insecticides are caused by an inhibition of acetylcholinesterases activities. However, in our earlier works, apart from cholinesterase inhibition, we demonstrated a liver injury [1, 2, 3]. A number of other authors described the changes in the liver parameters of animals treated with some organophosphate insecticide, but the mechanism of liver damage remains not fully understood [4, 5, 6].

Experimental data show no correlation between organ damage and the degree of organophosphate induced acetylcholinesterase inhibition, the main mechanism of its toxicity, suggesting the involvement of alternative mechanisms [7, 8]. Some reports, including our data, have suggested that reactive oxygen species (ROS) are involved in liver disturbances after treatment with organophosphate insecticides [9, 10]. Both the hyperproduction of ROS

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and the weakening of natural scavenging mechanisms have been implicated as contributors to oxidative stress and organ failure. Many authors have proposed the use of antioxidants, for example N-acetylcysteine, to reduce oxidative damage. N-acetylcysteine (NAC) is a well known artificial precursor of reduced glutathione (GSH), the key non-enzymatic antioxidant [11, 12, 13].

The present study was intended to examine if NAC changes the liver GSH level as well as influences ChE serum activity in rats intoxicated with chlorfenvinphos.

Material and Methods

The studies were conducted on male Wistar rats of 200±20 grams body weight. The rats were fed a standard diet and given water to drink ad libitum. The animals were divided into two groups. One group of rats was pretreated with 0.1% water solution of NAC. NAC was administered in drinking water 24h before intoxication. The control groups received oil intragastrically by stomach tube in the amount of 0.1ml/100g (I), immediately or after NAC pretreatment (II and VII). The experimental groups received oil solution of chlorfenvinphos, i.e. 2-chloro-1-(2,4-dichlorophenyl) vinyldiethyl phosphate (CVP) in a dose of 0.02 LD_{50} (III and VIII) or 0.1 LD_{50} (V and X) (LD_{50}=15 mg/kg b.w.) immediately or after pretreatment with NAC (IV, VI, IX, and XI). The rats received on average 35.60±3.23 mg of NAC/24h. One and 24 hours after intoxication with chlorfenvinphos (or after NAC pretreatment - group VII) the blood samples were collected, and livers were quickly removed and placed in iced 0.9% NaCl containing 0.16 mg/ml heparin. Our study was approved by the Local Ethical Committee.

Serum ChE activity was measured according to Ellman methods [14].

Liver for reduced gutathione determination was homogenized in ice-cold 5% metaphosphoric acid, centrifuged at 3000g, 4^0C for 10 minutes. Levels of hepatic GSH were measured using a 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 reagents 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.

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

Results

The results of this study demonstrated the changes in ChE serum activity and liver glutathione levels in the rats after administration of chlorfenvinphos at single doses.

ChE serum activity was inhibited at the 1st hour after treatment with chlorfenvinphos at a dose of 0.02 LD_{50} (by 25%), and with a dose 0.1 LD_{50} (by 50%). At the 24th hour of intoxication ChE activity returned to the control value after treatment with the lower dose and remained decreased after intoxication with the higher one. ChE serum activity did not change, statistically significantly, after pretreatment of rats with NAC. The pretreatment of rats with NAC also did not influence serum ChE activity in the intoxicated rats (Table 1).

The treatment of rats with chlorfenvinphos at a dose of 0.02 LD_{50} resulted in a decreased concentration of GSH in the liver of rats, at the 1st and 24th hours. In contrast, liver GSH concentration increased statistically significantly compared to the control group, throughout the experiment, after intoxication with chlorfenvinphos at a dose of 0.1LD_{50} (Tab 1). The pretreatment of rats with NAC did not change liver GSH levels statistically signficantly, but it resulted in increased GSH levels in the liver of rats intoxicated with chlorfenvinphos at a dose of 0.02 LD_{50}. Pretreatment with NAC did not influence, statistically significantly, liver GSH levels in rats intoxicated with a higher dose of chlorfenvinphos (Table 1).

Discussion

The present study demonstrated the dose-dependant inhibition of serum ChE activity after treatment of rats with chlorfenvinphos at a dose of 0.02 and 0.1 LD_{50}. At the same time we showed that pretreatment of rats with NAC did not prevent ChE inhibition caused by chlorfenvinphos intoxication. Organophosphate insecticides cause their effects by inhibiting AChE, leading to ACh accumulation. The symptoms of such intoxication are due to activation of muscarinic and nicotinic receptors [15, 16]. However, the low plasma level of ChE does not necessarily correlate with the severity of overstimulation [16]. NAC acts by modulating GSH metabolism or as an ROS scavenger. Thus, the results of our work do not seem to be unexpected.

Tripeptide GSH is one of the most important endogenous antioxidants. It plays a role as a sulfhydryl group provider for direct scavenging reactions. GSH is synthesized inside the cells through a biochemical pathway composed of several enzymes. During the reaction of hydrogen peroxide scavenging, GSH is oxidized to glutathione disulfide by the glutathione peroxidase. GSH can be regenerated from GSSG by glutathione reductase [17, 18].

In this study, an increase in the concentration of liver GSH contents was found at the 1st and 24th hour after treatment of rats with chlorfenvinphos 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 a dose of 0.02 LD_{50}, a decrease in GSH levels was observed. Many authors have reported that the exposure of an organism to the generated oxidative stress substances results in the enhanced level of GSH [18, 19, 20]. This increase is caused by the enhancement of its

Table 1. Serum ChE activity (U/l) and liver GSH concentration in rats intoxicated with chlorfenvinphos after NAC pretreatment.

Group	Serum ChE	Liver GSH
I control	1458.16±160.24 (n=15)	23.56±1.64 (n=7)
II NAC	1500.12±132.53 (n=8)	22.35±2.12 (n=8)
III CVP - 0.02 LD_{50}-1h	1098.26±102.19 ^{ab} (n=8)	18.68±1.21 ^{ab} (n=8)
IV NAC+CVP - 0.02 LD_{50}-1h	1120.60±99.45 ^{ab} (n=8)	24.45±2.23 ^{c} (n=8)
V CVP - 0.1 LD_{50}-1h	729.76±149.21 ^{abcd} (n=8)	70.15±5.08 ^{abcd} (n=7)
VI NAC+CVP - 0.1 LD_{50}-1 h	780.21±131.96 ^{abcd} (n=8)	72.30±7.12 ^{abcd} (n=8)
VII NAC-24 h	1400.34±123.99 ^{ef} (n=8)	24.23±1.66 ^{cef} (n=8)
VIII CVP - 0.02 LD_{50}-24h	1390.00±150.20 ^{ef} (n=8)	17.87±1.34 ^{abdefg} (n=8)
IX NAC+CVP - 0.02 LD_{50}-24h	1289.99±149.78 ^{ef} (n=8)	22.32±1.04 ^{cefgh} (n=8)
X CVP - 0.1 LD_{50}-24h	1100.95±110.12 ^{abefgh} (n=6)	77.77±4.79 ^{abcdeghi} (n=7)
XI NAC+CVP - 0.1 LD_{50}-24h	1187.65±98.73 ^{abefgh} (n=8)	74.22±6.98 ^{abcdghi} (n=8)

values expressed as means ± SD; n- the number of rats in the group; statistically significant in comparison with: a- I, b- II, c- III, d- IV., e- V, f- VI, g- VII, h- VIII, i- IX, j- X;

synthesis after induction of γ-glutamylcysteine synthetase and this mechanism is considered to be adaptative [19]. It 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 levels during oxidative stress may result from modification in synthesis and/or loss [21, 22]. 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 catalyzed by GPx, as mentioned above [18].

Furthermore, we found that pretreatment of rats with NAC restored GSH levels in the liver of rats intoxicated with chlorfenvinphos at a dose of 0.02 LD_{50}. However, it did not influence its level in the livers of rats intoxicated with a higher dose of insecticide.

NAC is a precursor of GSH. It acts as a ROS scavenger as well as increases GSH level. For many years, NAC has been widely used as a mucolytic drug; more recently, it has been used with good results in patients with septic shock, and is currently indicated for use against acute overdosing with paracetamol [11, 12, 13]. Salvemini et al. [23] have reported that NAC counteracted the diminished level of GSH which was caused by some GSH-depleting drugs. In the present work we demonstrated this action of NAC but only in acute intoxication with a lower dose of chlorfenvinphos. The higher dose of insecticide induces enhancement of liver GSH levels and for this reason we suppose a further increase caused by NAC is not required. For the production of GSH, the appropriate level of NADPH (which is produced by the pentose phosphate pathway) is required. The first and rate-limiting enzyme of this pathway is glucose-6-phosphate dehydrogenase (G6PDH). Zhang *et al*. [24] observed an 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 levels [15]. In the acute intoxication with organophosphate, some authors have observed the changes in the density of muscarinic receptors. Thus, the activation of muscarinic receptors seems to influence the activity of G6PDH. However, in acute intoxication with chlorfenvinphos we did not observe any influence of NAC on serum ChE activity.

In conclusion, the results reported here indicate that NAC influences the decreased level of GSH in the liver of chlorfenvinphos-intoxicated rats. The mechanism of this effect, in our opinion, is non-cholinergic.

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