A STUDY ON THE EFFECTS OF DIAZINON AND CARBARYL ON CHOLINESTERASE ACTIVITY BY AN ELECTROMETRIC METHOD IN RABBITS

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ABSTRACT

The aim of the study was to examine the effect of diazinon and carbaryl, and validate the efficiency of a modified electrometric method in measuring cholinesterase activity (ChE) in rabbits. Oral administration of female rabbits with diazinon at 35 mg/Kg, and with carbaryl at 500 mg/Kg, induced signs of toxicosis characteristic of cholinergic over stimulation. The signs were associated with significant decreases of plasma, erythrocyte and brain ChE activities with diazinon, but with carbaryl there were only significant decreases of plasma and erythrocyte ChE activities in comparison with control values. The extent of ChE inhibition in the erythrocyte correlated well with that of the brain in diazinon treated rabbits. The results suggested that the described electrometric method is simple, accurate and efficient in measuring the ChE inhibition caused by diazinon and carbaryl insecticides in rabbits.

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INTRODUCTION

Organophosphorus compounds such as diazinon are irreversible inhibitors of cholinesterase (ChE) (1, 2, 3, 4), while carbamates such as carbaryl are reversible inhibitors of ChE (2, 5, 6). Both of them cause cholinergic stimulation. The toxic signs result from accumulation of acetylcholine (ACh) at the central and peripheral nerve endings (1, 2, 3, 7). Cholinesterase is considered the target enzyme for diazinon and carbaryl toxicity, so it is considered to be the most important enzyme for diagnosing and assessing exposure to the effect of ChE inhibitors by measuring ChE activity in brain, erythrocyte and plasma (1, 2, 8). Numerous procedures are used to determine ChE activity in man and animals (9, 10). The electrometric method is one of the most widely used techniques to measure ChE activity (11). Numerous modifications of the electrometric method originally described by Michel (11) are the most commonly used with different animal species (9, 12). One of the most recent modifications was characterized by being simple and accurate and the time of assay is short (30 minutes) (12). The method has been used on a limited scale in mice (13), rats (14), cockerels (14), chickens (15), cattle (16), goat (17), fish (18) and man (19). The latest modifications of the electrometric method used suitable substrate (Acetylthiocholine iodide) with a sample (0.2ml) volume of and a short incubation time (20 minute) (19). However, the method needs further validation standardization to monitor any change in ChE activity in rabbits. Therefore, the present study was undertaken to evaluate the efficiency of the modified electrometric method in detecting ChE inhibition in rabbits.

MATERIALS AND METHODS

Female rabbits (1-2 Kg body weight) were used. They were housed under standard conditions with 10/14 hrs light/dark and 20-22 °C room temperature. The rabbits were randomly divided into three groups; of 5-7 animals for each group.

Group one was given diazinon (Devidyl 60/ EC Agrochemicals, India) orally, at 35mg/Kg (4).

Group two, given carbaryl (85% powder, Sociedad Anonima, DeAgroquimicos, Spain), orally at 500 mg/Kg (20).

Group three, given distilled water (control), orally at 2 ml/kg. Immediately after dosing each rabbit was kept in individual metallic cages for 2 hour to observe the occurrence of signs of muscarinic, nicotinic and CNS effects (21). These included salivation, lacrimation, defecation, frequent urination, piloerection, flat body appearance, gasping, tremor and convulsion. At the end of 2 hour period, blood samples were obtained after slaughtering the animals and collected in heparinized test tubes and immediately centrifuged at 300 rpm for 15 minute to separate plasma and erythrocytes. The skull was immediately opened by surgical blade to obtain the whole brain. All samples were stored immediately at -
20 C° for later measurement of ChE activity. The electrometric method (12) as modified later by (19) was used to measure ChE activity. Statistical analysis: Enzyme inhibition was statistically analyzed by one way analysis of variance following the least significant difference test. (22). Linear regression was used to determine the correlation between the blood ChE (plasma and erythrocyte) inhibition and brain ChE inhibition. The level of significance was P≤ 0.05.

RESULTS
Diazinon and carbaryl in the rabbits induced signs of cholinergic toxicity and tremor within 20-30 minutes. The toxic signs were severe salivation, lacrimation, frequent urination, piloerection, defecation, ataxia, gasping, convulsion, and flat body appearance. Rabbits dosed with diazinon manifested more potent signs and the onset of action was short in comparison with the carbaryl treated group. Oral dosing of rabbits with diazinon at 35mg/kg and carbaryl at 500mg/kg inhibited plasma ChE by 64% and 36%, respectively in comparison with control values (Table 1). Diazinon also significantly inhibited erythrocyte ChE by 27% and that of the brain by 54% in comparison with the control values (Table 1). Inhibition of erythrocyte ChE by Carbaryl was also significant by 47% but brain ChE inhibition not significant in comparison with control value (Table 1).

The inhibition of brain ChE activity positively correlated with inhibition of erythrocyte ChE activity in the diazinon treated group (r = 0.88) (Fig 1). However, in carbaryl treated group inhibition of brain ChE did not correlate well (r=0.512 and 0.269) with those of plasma and erythrocytes, respectively (Fig 2).

Table 1: ChE inhibition in rabbits dosed orally with ChE inhibitors

<table>
<thead>
<tr>
<th>Cholinesterase inhibitor (mg/kg, orally)</th>
<th>ChE activity (Æ pH/30 min) inhibition</th>
<th>% inhibition</th>
<th>ChE activity (Æ pH/30 min)</th>
<th>% inhibition</th>
<th>ChE activity (Æ pH/30 min)</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>Brain</td>
<td>Plasma</td>
<td>Erythrocyte</td>
<td></td>
<td>Erythrocyte</td>
<td></td>
</tr>
<tr>
<td>0 (control)</td>
<td>0.13 ± 0.007</td>
<td>0.36 ± 0.007</td>
<td>0.15 ± 0.005</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diazinon 35 mg/kg.b.w</td>
<td>0.06 ± 0.004*</td>
<td>54</td>
<td>0.13 ± 0.006*</td>
<td>64</td>
<td>0.11 ± 0.005*</td>
<td>27</td>
</tr>
<tr>
<td>Carbaryl 500 mg/kg.b.w</td>
<td>0.11 ± 0.01</td>
<td>15</td>
<td>0.23 ± 0.01*</td>
<td>36</td>
<td>0.08 ± 0.005*</td>
<td>47</td>
</tr>
</tbody>
</table>

N= 5-7 rabbits/group
The measure values represent mean ± SE.
Significantly from the spectra control value P ≤ 0.005.
Figure 1: Correlation between brain cholinesterase and plasma or erythrocyte cholinesterase activity in rabbits dosed orally with diazinon.

Figure 2: Correlation between brain cholinesterase and plasma or erythrocyte cholinesterase activity in rabbits dosed orally with carbaryl.

**DISCUSSION**

The signs of toxicosis seen in rabbits after oral administration with diazinon and carbaryl were cholinergic over stimulation as found in rodents (21, 23, 24, 25). Organophosphorous and carbaryl compound inhibit ChE leading to accumulation of toxic levels of ACh at the nerve endings causing parasympathetic
over stimulation (1, 2, 5, 7, 26). The inhibition of ChE in plasma, erythrocyte and brain by diazinon as well as inhibition of plasma and erythrocyte ChE by carbaryl were according with the findings of previous studies in which the insecticides such as organophosphorous and carbamates were found to inhibit blood and brain ChE (2, 3, 25). The non significant inhibition of ChE in brain with carbamates may be attributed to the reversible inhibition of ChE by carbamates (2, 5, 6). Carbamylated ChE reactivates spontaneously faster than the diazinon (2, 10). This was evident as many severe signs of toxicosis with carbaryl disappeared before the end of the observation period (2h). ChE activity in blood and brain is useful for diagnosis of toxicity and monitoring exposure of man and animals to ChE inhibitors (9, 10). In the present study, the modified electrometric method (12), as a further modified (19), was applied to measure the ChE activity in rabbits dosed with diazinon and carbaryl. This method has been applied in various animal species (12, 14, 15, 16, 17, 18, 19). The present study further supports and validates these applications as being sensitive, simple, and efficient and the overall assay time (~30) is shorter than that of Michel's method.

There was good correlation between the level of erythrocyte ChE inhibition and the severity of the symptoms of diazinon and carbaryl. However, the inhibition of erythrocyte ChE activity may reflect the inhibition of ChE in the brain (10, 26). The present study also showed a positive correlation between the inhibition of brain ChE activity and that of erythrocyte in rabbits dosed with diazinon. A similar result was reported previously in rats (27, 28), intoxicated with organophosphorous insecticides.

In conclusion, diazinon and carbaryl inhibit ChE in plasma and erythrocyte. The recently modified electrometric method was simple, efficient and rapid for measuring the blood and brain ChE activities in rabbits after exposure to organophosphorous and carbamate insecticides.

REFERENCES