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Inhibition of Acetyl Cholinesterase Activity Farmers Exposed to Organophosphate Pesticides in Bushehr, Iran

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Abstract: Organophosphates (OPs) are widely used in Palm plantations as pesticides by farmers from bushehr province, Iran. They are irreversibly bound to cholinesterase, causing the phosphorylation and deactivation of acetycholinesterase (AChE). The activity of Acetyl cholinesterase (AChE) can be used as the best potential biomarker of exposure to organophosphates. In this study, the Acetyl cholinesterase enzyme was evaluated in whole blood samples of farmers from Ab-pakhsh community in *Bushehr*. Results showed that the AChE activity were significantly lower than control group ($P<0.05$). This study showed that AChE inhibition provide a good biomarker of exposure to OPs pesticides in human population. In addition, the authors recommend use of protective equipments and the adoption of other safety practices during fieldwork.

Key words: Organophosphates · Acetyl cholinesterase · Safety practices

INTRODUCTION

Organophosphorous (OP) compounds are one the most important and diverse classes of pesticides. They are used as insecticides and, to a lesser extent, as herbicides. Most of them, are derivatives of phosphoric and thiophosphoric acids [1]. Inhibition of acetylcholinesterase (AChE) is considered the major mechanism of organophosphate toxicity [2]. Cholinesterase inhibitors (CEI), such as carbamates and organophosphates (OPs), have been widely used and studied since the 19th century [3]. CEIs are widely used as insecticides and pesticides and some of them (extremely toxic) are manufactured and stored as biological weapons that cause Gulf War syndrome in soldiers [4]. Anti cholinesterase activity induced by an OP ester, have two steps of the formation of enzyme inhibitor complex and the phosphorylation of the enzyme which are presented in Figure 1 [5].

While the AChE remains phosphorylated, it is unable to perform its natural function of hydrolyzing ACh. This results in over activity at sites of cholinergic neurotransmission and can cause disturbance in numerous body functions [2, 6]. Therefore, acetyl cholinesterase statuses are used as potential biomarkers of organophosphate exposure or intoxication [7].

Bushehr province located in the south of Iran, with a long coastline onto the Persian Gulf is surrounded by abundance of waters sources and fertility of soil, is a rich and well-endowed land for the agricultural developing. Organophosphates (OPs) are widely in Palm plantations used as pesticides by farmers at this province. Since there is an increasing international public health concern about the neurotoxic potential of organophosphate (OP) pesticides, in this study the occupational exposure to OP pesticides and their effects on the concentration of cholinesterases were investigated in farmers from Ab-pakhsh in Bushehr- Iran.

MATERIALS AND METHODS

Materials: Acetylthiocholine iodide (ASCh), 5, 5'-dithio-bis-2-nitrobenzoic acid (DTNB, Ellman's reagent), Triton X-100 were obtained from Sigma (Deisenhofen, Germany), ethopropazine hydrochloride from Aldrich (Steinheim, Germany), $Na_2HPO_4 .2H_2O$, KH_2PO_4, $NaHCO_3$, HCl from Merck (Darmstadt, Germany).

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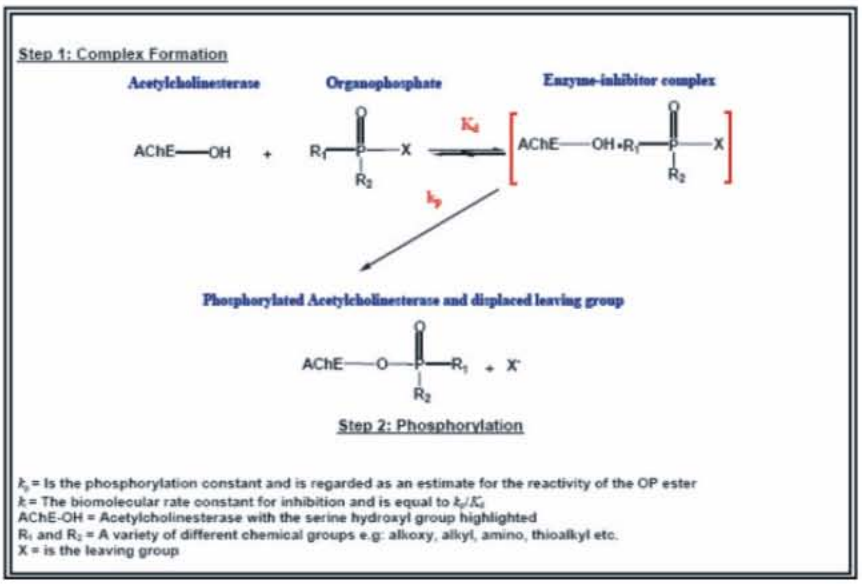

Fig. 1: A Schematic equation for OP inhibition of AChE. Two steps of the formation of Enzyme- inhibitor complex and phosphorylation of the enzyme, see in Figure 1 [5]

Reagents

Phosphate Buffer (PP, 0.1 mol/l, pH 7.4): A 17.8 g of $Na_2HPO_4 \cdot 2H_2O$ and 2.72 g of KH_2PO_4 was dissolved in 1000 and 200 ml of distilled water, respectively. The two solutions were mixed together until reaching the pH to 7.4, the PP was then filtered by HA filter, Millipore and stored at 4°C.

Color Reagent (DTNB, 10 mmol/l): A 396.3 mg DTNB was dissolved in 100 ml of PP by magnetic stirring and stored in 5 ml aliquots at -20°C.

Substrate (ASCh, 28.3 mmol/l): A 82.24 mg acetylthiocholine was dissolved in 10 ml of distilled water and stored in 1 ml aliquots at -20°C.

Ethopropazine (6 mmol/l): A 20.94 mg ethopropazine was slowly dissolved in 10 ml of 12 mmol/l HCl and stored in 500 µl aliquots at –20°C.

Diluting Reagent for Whole Blood Samples: A 300 µl Triton X-100 was added to 1000 ml of PP and stored in an amber bottle at 4°C.

Sample Preparation: The whole blood samples were collected from farmers in Ab- pakhsh, bushehr with, who exposed to OP pesticide follow the spraying. The blood samples were also collected from a control groups who had never done farm work and had never been in contact with toxic substances. Samples collected in EDTA-tubes were diluted by adding 200 µl of blood to 20 ml ice-cold diluting reagent (v/v: 1/100). After careful mixing, the samples were immediately frozen in 1 ml aliquots at -20°C and kept until analysis. Prior to analysis, the whole blood dilutions were thawed by gentle shaking of the vials in cold water and kept on ice until analysis.

Apparatus: The AChE activity was measured with a CECIL CE 7250 spectrophotometer (Bio Aquarius, England).

Procedure: AChE activity, was determined according to the Ellman kinetic method, modified by Worek *et al.* [2]. The absorbencies were measured at 436 nm and 37°C using polystyrol cuvets. The detailed procedure is given in Table 1.

RESULTS

AChE Activity in Whole Blood: AChE Activity in farmers from control and Ab-pakhsh groups are summarized in Table 2.

The mean of AChE activity in samples were significantly lower ($P<0.05$) than mean of activity in control group. It was 4314.646 and 4055.111 (µmol/l/min) in samples from Ab-pakhsh and control groups, respectively.

Table 1: Standard procedure for the determination of AChE activitiy

	AChE
Mix in polystyrol cells: Phosphate buffer (pH: 7.4/ 0.1 mol)	2.000 ml
DTNB (10 mmol/l)	0.100 ml
Ethopropazine (6 mmol/l)	0.010 ml
Hemolysate (whole blood 1:100)	1.000 ml
Equilibrate at 378°C for 10 min, then add: ASCh (28.4 mmol/ l)	0.050 ml

The color development was recorded for 10 min at 37°C and 436 nm ($\varepsilon = 10.6 \times 10^3$) nm against water blank

Table 2: AChE activity (μmol/l/min), in whole blood in farmers and control groups

Activity		
Groups		AChE Activity (μmol/l/min)
Control N:30	Mean	4314.646
	Std. Deviation	890.1346
Ab-pakhsh group N:33	Mean	4055.111
	Std. Deviation	1078.006

DISCUSSION

In this study, AChE activity in farmers exposed to OPs was significantly lower than mean of activity in control group, which is in accordance with results reported from other countries. Rendon *et al.* [8] determined AChE activity in four rural communities of farmers from Campeche, Mexico during insecticide use. The AChE activity was significantly lower than the mean of activity in a control group. In addition, the AChE activities in farmers from Bushehr were significantly lower than mean of activity in control group ($P<0.05$).

Previous studies showed that AChE activity provide good biomarkers of exposure to OPs in field studies with human populations. These biochemical parameters usually decrease in sprayers and workers at the end of work.

The full return AChE activity to normal depends on its re-synthesis that limited to about 0.8% per day [9].

According to our observations, farmers were applied OP pesticides on their farms without taking proper protective measures. They exposed to highly poisonous OPs pesticides and inhaled substantial amounts of these compounds during spraying. They did not even properly wash their hands and faces after spraying pesticides. Some farmers not only did not dispose the empty pesticide containers, but also, sometimes used the containers for storing food. Therefore, they need to be aware of risk of exposure to these poisons. The authors recommend use of protective equipment and adoption of other safety practices during fieldwork, increase knowledge about the risks of OP pesticides, safety practices.

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