In Vitro Inhibition of Blood Cholinesterases by the Organophosphate Dichlorvos in Typ-2 Diabetic Patients

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Abstract

Background: Cholinesterases may undergo metabolic alterations due to type 2-diabetes mellitus and their susceptibility to in vitro inhibition by organophosphate insecticides is not known. The aim of the study was to examine in vitro inhibition of plasma and erythrocyte cholinesterase activities in type-2 diabetic patients by the organophosphate insecticide dichlorvos. **Methods:** Plasma and erythrocyte cholinesterase activities were measured in 32 type-2 diabetic patients and 32 apparently healthy subjects by the spectrophotometric Ellman method. The 10-minute cholinesterase-inhibitor incubation method was used to examine the in-vitro inhibitory effects of dichlorvos (0.5 and 1 μM) on plasma and erythrocyte cholinesterase activities in type-2 diabetic patients vs. control healthy subjects. **Results:** Plasma and erythrocyte cholinesterase activities in type-2 diabetic patients were significantly higher than those of the apparently healthy control subjects. Dichlorvos at 0.5 and 1 μM in vitro significantly inhibited plasma and erythrocyte cholinesterase activities in both control and diabetic subjects. However, the percentages of blood cholinesterase inhibition in diabetic patients were more than those of respective control values.

Conclusion: Patients with diabetes could be more sensitive to toxicity by cholinesterase inhibitors. Accordingly, caution should be practiced in patients using cholinesterase inhibitors.

Keywords*: cholinesterase, diabetes, dichlorvos, in vitro inhibition, organophosphorus*

Introduction

Diabetes mellitus (DM) is a heterogeneous metabolic abnormality characterized by the occurrence of hyperglycemia due to deficiency and/or loss of insulin secretion.[1,2] It may induce structural and functional tissue injuries with complications in the cardiovascular and neuronal systems.[1,3] Cholinesterases (ChE) are a group of enzymes which hydrolyze acetylcholine into choline and acetic acid, which is an essential metabolic process to restore cholinergic neurotransmission.[4-6] True ChE is present at cholinergic synapses in the brain, autonomic ganglia and at the neuromuscular junctions, whereas pseudo ChE is mainly found in the plasma and liver.[4,5]

Several studies have shown that DM, regardless of the cause, may modulate ChE activities in the blood and may cause an imbalance between true and pseudo ChE activities in neuronal tissues and the blood.[7-9] Studies reported either an increase[9-13] or decrease[14-16] in plasma (serum) or erythrocyte ChE activities. Furthermore, Garcia et al.[17] reported that reduced muscular ChE activity is attributed to streptozotocin-induced hyperglycemia in mice. Changes in enzymatic activities in patients could be related to high glucose levels in the blood regardless of the associated antidiabetic drug therapy.[1,3,6,9,13]

Based on these potential modulatory effects of DM on ChE activities, there are concerns about the susceptibility of diabetics to poisoning by ChE inhibitors.[18-21] Organophosphates were reported to disrupt glucose homeostatsis as well other enzymatic pathways involved in metabolism of carbohydrates, fats and proteins.[19,20] The primary mechanism of toxic action of organophosphates is inhibition of ChE activities at the nerve endings causing cholinergic overstimulation.[5,22,23] One important tool to assess the potential risk of

organophosphates in man and animals is monitoring blood ChE inhibition in vitro.[24-28] We anticipate that changes in blood ChE activities in diabetic patients[7-9] might alter the in vitro susceptibility of blood ChE to organophosphate insecticides. The purpose of the present study was to examine in type-2 diabetic patients vs. healthy controls the plasma and erythrocyte ChE inhibition in vitro by the organophosphate dichlorvos which is used as an insecticide in agriculture and in veterinary practice.[29]

Materials and Methods

Subjects of the study were chosen from Azadi Hospital, Duhok, Iraq, between September 2017 to March 2018. The age of the participants included in this study ranged from 25 to 65 years. A brief explanation about the main goals of the study was given to the participants following the invitation to take part in the study. Written consent forms were obtained from all the participants. They were asked to read the consent form and for those who could not read, assistance was provided.

According to a questionnaire form, data were collected from all enrolled type-2 diabetic patients (n=32) and apparently healthy control subjects (n=32, 16/gender/group). Healthy subjects were selected from non-smokers, alcohol-free, having no history of chronic diseases and with a fasting glucose level (60-125 mg/dL). A patient was diagnosed having type-2 diabetes on the basis of WHO criteria.[30] Blood glucose levels were determined by using conventional certified assay recommended by local health authorities.

Five mL of venous blood sample was collected from each subject. The plasma and the erythrocytes were separated using a cooling centrifuge (4 °C) at 3000 rpm, for 15 minutes. The plasma and erythrocyte samples were stored at -30 °C pending analysis within one week.

Plasma and erythrocyte ChE activities were determined by the Ellman's spectrophotometric method,[31] which is based on the production of thiocholine as a result of hydrolysis of acetylthiocholine. The development of yellow color product was measured spectrophotometrically at 410 nm.

The method of incubation of organophosphate with plasma or erythrocyte aliquots was used to measure the in vitro inhibition of plasma and erythrocyte ChE activities by the organophosphate insecticide dichlorvos (Sigma Aldrich, Germany) as described earlier.[25-27] Briefly, the aqueous dilution of the insecticide was added to the enzymatic reaction mixtures of the plasma or erythrocytes to obtain final concentrations in the reaction mixtures at 0 (base-line control), 0.5 and 1 μM. The reaction mixtures containing the insecticides were incubated at 37°C for 10 minutes to induce enzyme inhibition. Thereafter, the residual ChE activity in the mixture was measured spectrophotometrically.[31] The percentage of ChE inhibition in the plasma or erythrocytes was calculated:

% ChE inhibition = ChE activity (without dichlorvos) – ChE activity (with dichlorvos)/ChE activity (without dichlorvos) × 100

Unpaired Student's t-test was used to analyze statistical significant difference of ChE activities between the diabetic and control subjects, using the statistical package Past 4.03 (https://folk.universitetetioslo.no/ohammer/past). The level of statistical significance was set at $P < 0.05$.

Results and Discussion

Plasma and erythrocyte ChE activities in type-2 diabetic patients were significantly higher than those of the apparently healthy control subjects regardless of the sex and age groups (Table 1).

Table1. Mean ± SD of plasma and erythrocyte cholinesterase (ChE) activities (mM/min) in type- 2 diabetic patients

Groups	Plasma ChE (n=32)	Erythrocyte ChE (n=32)
Control	0.263 ± 0.046	0.269 ± 0.054
Diabetic	0.304 ± 0.072*	0.342 ± 0.064*

* Significantly different from the corresponding control value, $p \leq 0.05$.

This finding is in accordance with previous reports in which diabetic patients had increased ChE activity in the serum and/or erythrocytes.[9,11-13] In the present study, the abnormally high blood ChE activities correlated well with the hyperglycemic status of the patients (data not shown). Experimental findings in rats[32] and mice[17] suffering from streptozotocin-induced diabetes further supports the notion that diabetes modulates ChE activity. The mechanism of increased ChE activity in diabetic patients is not fully clear at present. Usually, diabetic patients have high oxidative stress indices in the blood which might be accompanied with cholinergic dysfunction[1,33-35] It is possible; therefore, that oxidative stress status in the patients is the contributing factor to such an enzymatic change. Correspondingly, experimentally induced oxidative stress in 2-week old chicks was found to increase the susceptibility of the animals to organophosphate poisoning, which acts primarily to inhibit cholinesterases in the neuronal tissues, neuromuscular junctions as well in the plasma and erythrocytes.[18] Further evidences in rats suggest a modulatory role of oxidative stress on ChE activity.[35] Induction of free oxygen radicals can increase the toxicity of pesticides including the organophosphates.[36] Erythrocyte membranes are sensitive to oxidative damage due to their high polyunsaturated fatty acid content as well as high oxygen and hemoglobin concentrations.[33,35] Therefore, it is speculated that oxidative stress in erythrocytes can modulate their structural and functional aspects and render them more susceptible to ChE inhibitors.[33,37] However, a direct modulatory effect of high blood glucose status on the enzyme cannot be ruled out.[7,38] Furthermore, increased ChE activity was attributed to changes in glucose and lipid metabolism in diabetic patients.[39]

In contrast to our study and those of others cited previously and evidences presented above, other studies reported reduced blood ChE activities in diabetic patients.[14-16] The reason for this discrepancy is not clear at present. However, contributing factors to the variations in the results might include, but not limited to, age, stage of the illness, medications taken, and the extent of oxidative stress induced in the patients.[1,2,7,33,37].

An important finding of the present study is the increased in vitro inhibition of plasma and erythrocyte ChE activities by the organophosphate insecticide dichlorvos. Dichlorvos at 0.5 and 1 µM in vitro significantly inhibited plasma and erythrocyte ChE activities in both control and diabetic subjects (Table 2). This finding correlates with previous reports on in vitro inhibitory effects of organophosphates on blood cholinesterases.[25-27]

Table2. Percentages of in vitro inhibition of plasma and erythrocyte cholinesterase (ChE) activities by dichlorvos 0.5 and 1 µM

Group	Plasma ChE (n=32) % inhibition		Erythrocyte ChE (n=27) % inhibition	
	0.5µM	1µM	0.5µM	1µM
Control	28 ± 12	38 ± 13	22 ± 14	29 ± 10
Diabetic	43 ± 16*	46 ± 17*	34 ±18*	45 ± 17*

Values are Mean ± SD.

* Significantly different from the respective control value, $p \leq 0.05$.

However, the percentages of plasma and erythrocyte ChE inhibitions in type-2 diabetic patients were higher than those of respective control values (Table 2). Inhibition of neuronal ChE activities is the primary target of organophosphate poisoning.[4,5,22] The resultant cholinergic overstimulation produce signs of poisoning characterized by nicotinic, muscarinic and central nervous system effects.[4,5,18,22,23] In this context, organophosphates in general inhibit plasma (serum) and erythrocyte ChE activities to various extents, mostly more than 30%.[4,5,22] Therefore, reduced blood ChE activity is a confirmatory biomonitoring test to diagnose organophosphate insecticides poisoning.[4,5,22,40] The in vitro plasma or erythrocytes-organophosphate incubation test is a versatile and robust procedure acts as a predictor of potential toxicity of organophosphate insecticides. [24-28] This was accomplished in the present study (Table 2). These findings of in vitro blood ChE inhibition by dichlorvos strongly suggest the increased susceptibility of the diabetic patients to organophosphate poisoning. However, an interesting finding reported that dosing of ChE inhibitors in mice decreased hyperglycemia and consequently the incidence of diabetes.[41] Based on the findings of the present study and those of others[13] utmost consideration should be given to increases in blood ChE in predicting risk of future type-2 diabetes with the possibility of increased susceptibility to ChE inhibitors. Therefore, further studies are needed to examine the in vitro ChE inhibition in diabetic patients on anticholinesterase medications or those on prolonged antidiabetic medications.

In conclusion, patients with type-2 diabetes could be more sensitive to toxicity by ChE inhibitors. Accordingly, caution should be practiced by these patients using anticholinesterase medications or those coming in contact with organophosphate insecticides.

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Ethical Clearance: Ethical approval and informed consent

The study was approved by the Postgraduate Committee of the College of Medicine, Duhok University and by the Local Research Ethics Committee at the Duhok Health Office, Duhok, Iraq.

Competing Interests : The authors declare that they have no competing interests.

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