Evaluation of acetylcholine esterase activity in the blood of workers exposed to organophosphate and carbamate insecticides by an electrometric method

Aydin S. Ahmed
Medical Laboratory Sciences Department -College of Technology- Kirkuk
E-mail: aydinfedakar@yahoo.com
Received date: 1/7/2013 Accepted date: 6/10/2013

Abstract

*Introduction:*Organophosphate and carbamate insecticides pose major environmental pollution problems and health hazards to people and animals. These insecticides inhibit cholinesterase (ChE) activity in the nervous tissues and neuromuscular junctions. The measurement of blood ChE is a useful tool for monitoring exposure to organophosphate and carbamate insecticides. The purpose of the present study was to use a modified electrometric technique for measuring blood ChE in workers exposed to the organophosphate and carbamate insecticides in Kirkuk, Iraq.

Method: A modified electrometric method was used to measure ChE activity in the whole blood of male workers (n = 40) exposed to organophosphate and carbamate insecticides, for a duration of not less than six years. Healthy male volunteers (n =12) not exposed to insecticides served as controls. Following in vitro inhibition of pseudo cholinesterase by quinidine sulfate, true cholinesterase activity was estimated in the blood of the subjects. After in vitro addition of the organophosphate (chlorpyrifos and methidathion, 0.5 and 1 µM) and carbamate (carbaryl, 5 and 10 µM) insecticides to the reaction mixtures, inhibitions of blood ChE were also determined.

Results: Mean values of ChE activities (ΔpH/20 min) in the whole blood of healthy non-exposed subjects and insecticide-exposed workers were 1.41and 1.2, respectively. Whole blood ChE activities of the exposed workers was significantly lower than those of healthy individuals.

Conclusions: These findings indicate the usefulness of the modified electrometric method for monitoring blood ChE activity in insecticide-exposed workers and there was a significant effect of these Organophosphate and carbamate insecticides on the activity of Ach esterase in workers blood. .

Keywords: Cholinesterase, organophosphate, workers, electrometric method.

تقييم نشاط الخميرة الاستيل كولين في دم العمال المعرضين لمبيدات المركبات الفوسفات العضوية والكارباميت بطريقة المقياس الالكتروني

ايدن صديق احمد
قسم التحليلات المرضية الطبية. الكلية التقنية
تاريخ استلام البحث:2013/7/1 تاريخ قبول البحث:2013/10/6

الخلاصة

تساهم المركبات العضوية الفوسفوتية والكارباميت في مشاكل التلوث البيئي والمخاطر الصحية في الأنسان والحيوانات حيث تعمل هذه المبيدات على تثبيط نشاط خميرة الأسيتيل كولين استيريز في الأنسجة العصبية ومواقع ارتباط الأعصاب بالعضلات حيث يعتبر قياس نشاط هذه الخميرة في الدم مؤشرا لتأثير هذه المركبات عند التعرض اليها. تهدف الدراسة الى استخدام طريقة محورة لقياس نشاط خميرة الأسيتيل كولين استيريز في دم العمال المعرضين الى هذه المبيدات في مدينة كركوك . خضعت للدراسة اربعون عاملا من المعرضين الى مبيدات المركبات العضوية الفوسفوتية والكارباميت لفترة عمل لا تقل عن ست سنوات يتراوح اعمارهم بين 20–50 سنة مع 12 شخص من الأصحاء من نفس الفئة العمرية الغير معرضين الى المبيدات.

استخدمت الطريقة المحورة(المقياس الألكتروني) لقياس نشاط الخميرة في دم العمال. تم قياس نشاط خميرة الأسيتيل كولين استيريز في دم الخاضعين للدراسة بعد التثبيط التجريبي لخميرة الأسيتيل كولين الكاذب بواسطة سلفات الكويرين . أدلت النتائج الى ان متوسط قيم نشاط خميرة الأسيتيل كولين استيريز(ΔpH)في 20 دقيقة حضن في دم الأشخاص الأصحاء كانت 1.41 وانما نقصت تلك القيم في دم العمال حيث اصبحت 1.2 . اي ان هناك نقصان معنوي ($p<0.5$) في نشاط الخميرة. استنتجت من الدراسة بان استخدام الطريقة الألكترونية مفيدة لقياس نشاط هذه الخميرة وان المركبات العضوية الفوسفوتية والكارباميت لها تأثير معنوي على نشاط خميرة الأسيتيل كولين استيريز.

الكلمات الدالة: خميرة الأسيتيل كولين، مبيدات، مركبات الفوسفات، عمال.

Introduction

Acetylcholinesterase (AChE) is an important enzyme present in the synaptic clefts of the central nervous system of living organisms [1]. It hydrolyses the neurotransmitter acetylcholine and facilitates the proper functioning of muscular system and it is used as a marker for cholinergic neural function [2]. AChE has been subject of keen interest for several decades and the detailed studies carried out in the past have revealed that the AChE activity could be significantly inhibited by organophosphorus (OP) pesticides used in veterinary practice, agriculture, medicine, industry and chemical warfare agents [4,3].Organophosphate and carbamate insecticides are widely used in public health, veterinary practice, and in agriculture [5,6]. They pose major environmental pollution problems and health hazards to people and animals [4,7-9]. These insecticides inhibit cholinesterase (ChE) activity in the nervous tissues and neuromuscular junctions, causing an accumulation of acetylcholine at the nerve endings which subsequently produces signs of toxicosis characterized by nicotinic, muscarinic, and central nervous system effects [8,10- 11]. Various colorimetric and electrometric (potentiometric) methods are available to determine blood cholinesterase activity [12,13]. One of the main methods for measuring blood cholinesterase activity is the electrometric method which is based on the hydrolysis of acetylcholine and the production of acetic acid that subsequently decreases the pH of the reaction mixture [14]. Normal reference values of plasma and erythrocyte ChE activities of sheep, goats and cattle [15] and treated with organophosphate insecticides [16] are reported by using the above mentioned electrometric method. The method has been also used in apparently healthy human volunteers to report their normal reference values of blood cholinesterases [17]. Few reports are found in the literature on the use of the modified electrometric method for monitoring blood cholinesterase activity of workers exposed to inhibitors of this enzyme [18]. The purpose of our study was to further evaluate and apply the modified electrometric method for measuring blood ChE activities in workers exposed to organophosphate and carbamate insecticides in Kirkuk, Iraq .

Subjects and Methods

Forty male workers, in contact with and exposed to carbamate and organophosphate insecticides daily and for a period of working duration not less than 6 years, were included in the study. Their ages ranged between 20-50 years .Apparently healthy volunteers (n=12), who had no history of exposure to anti ChE insecticides for at least six months before blood sampling served as controls. The volunteers were from Kirkuk province, Iraq. Their consents

were obtained for the blood examination. The modified eletrometric method of Mohammad (2007) was used to determine whole blood ChE activity. For a typical assay condition, the reaction mixture in a 10-ml beaker contained 3 ml distilled water, 0.2 ml whole blood, and 3 ml pH 8.1 barbital-phosphate buffer.The pH of the mixture (pH1) was measured with a glass electrode using a pH meter (Consort, Belgium) before 0.1 ml of aqueous solution of the substrate acetylcholine iodide (7.1%) was added to the reaction mixture that was incubated at 37°C for 20 minutes. At the end of the incubation period, we measured the pH of the reaction mixture (pH2). The enzyme activity was calculated as follows:

• ChE activity (ΔpH/20 minutes) = (pH1 – pH2) -ΔpH of blank.

The blank was without the blood aliquot. The barbital-phosphate buffer solution consisted of 1.24 g sodium barbital (BDH), 0.163 g potassium dihydrogen phosphate (Merck, Germany), and 35.07g sodium chloride (BDH) dissolved in one liter of distilled water. The pH of the buffer was adjusted to 8.1 with 1 N HCl [17]

In vitro ChE Inhibition by Organophosphate (Chlorpyrifos and Methidathion) and Carbamate (Carbaryl) Insecticides: Pooled blood were collected from 6 male volunteers. The method of inhibitor-ChE incubation was used to cause in vitro inhibition of ChE activities in the blood sample by chlopyrifos (40%, VAPCO, Jordan) and methidathion (50%,Agricultural chemicals Manufacturing Enterprise, Jordan) and by carbaryl (85%, SociedadAnonima DeAgroquimicos, Spain) [16] . The insecticides were prepared in distilled water and individually added in a volume of 0.1 ml to the reaction mixtures of the whole blood. The final reaction volumes in control and inhibited samples remained the same (6.3 ml) by using 2.9 ml of distilled water instead of 3 ml. The final concentrations of chlopyrifos and methidathion in the reaction mixtures were 0.5 and 1 µM; final concentrations of carbaryl were 5 and 10 µM. Control reaction mixtures did not contain any insecticide, and they were used for measurement of base-line ChE values. The reaction mixtures were incubated at 37ºC for 10 minutes [16]. Thereafter, the residual ChE activity in the mixtures was measured as before. The% of enzyme inhibition was calculated as follows:

• % ChE inhibition= [ChE activity (without insecticide)-ChE activity (with insecticide)/ChE activity (without insecticide)] X 100

Statistics

The significance of ChE inhibition in the plasma or erythrocytes of each subject was statistically evaluated using unpaired Student's t-test. The level of significance was at $P < 0.05$.

Results

Tables 1 shows the mean, SD, and related statistics for whole blood ChE activities in apparently healthy subjects (non-exposure group) and workers exposed to insecticides. Whole blood ChE of the workers was significantly below that of the control group (Table 1) .

Table (1): Cholinesterase Activities (ΔpH/20 minutes) in the Blood of healthy volunteers and workers exposed to insecticides

Parameter	Normal subjects' blood	Workers 'blood
No.	12	40
Mean	1.44	1.12
SD	0.14	0.13
Range	1.2-1.83	1.08-1.1

The insecticides (chlorpyrifos, methidathion, and carbaryl) in a concentration-dependent manner variably inhibited bloodChE activities in vitro (Table 2).

Table (2): In vitro inhibition of human blood Cholinesterase Activities by chlorpyrifos, Methidathion and Carbaryl.

Insecticide conc.(µM)	ΔpH/20 minutes	Inhibition percentage
Baseline (0)	1.23± 0.105	0
chlorpyrifos		
0.5	1.13± 0.046	8
1	0.86± 0.126*	30
Methidathion		
0.5	1.03± 0.132*	16
1	0.86± 0.126*	70
Carbaryl		
0.5	0.53± 0.104*	57
1	0.43± 0.072*	65

***=statisticall significant (p<0.05)**

Discussion

This study introduces (for the first time) normal ChE activities of the blood of apparently healthy male and workers who exposed to organophosphate and carbamate insecticides in Kirkuk province (Iraq) as determined by a simple modified electrometric method. It is in agreement with the findings of Ahmed and Mohammad, 2007[18] in a different Iraqi region (Mosul).

The widespread use of pesticides in agriculture results in continuous exposure of human populations. Agriculture workers are prone to long-term exposure to relatively low levels of organophosphate agents. These workers are daily exposed, use little protection due to cultural and economic reasons, and underestimate the toxicity of organophosphate. Poisoning has frequently resulted from the use of organophosphorus pesticides, the compounds usually

having been absorbed dermally or by inhalation during application or during subsequent work in the fields.

Measurement of blood and tissue cholinesterase activities is a useful tool for monitoring exposure to organophosphate and carbamate insecticides and diagnosing their poisoning [10,19]. Usually a 20-30% decrease in serum cholinesterase activity suggests exposure to anticholinesterases [10]. More than 50% inhibition of cholinesterase activity supports the diagnosis of poisoning and indicates a hazardous condition [11].

Based on the findings of the present study and others [16,18,20], it can be assumed that the present electrometric method could have practical applications in human to detect ChE inhibition following exposure to anti-ChE insecticides. This method has a short one step-incubation time and it is sensitive enough, cheap and simple. The normal reference range values of plasma, erythrocyte and whole blood ChE activities of the healthy human volunteers have been reported using the presently described electrometric method [17, 20].

In vitro inhibition of whole blood ChE activities by chlorpyrifos, methidation (organophosphates), and by carbaryl (a carbamate) is in agreement with the reported anti ChE actions of these insecticides [16,17,21, 22]. These results and previous in vitro and in vivo ChE inhibition studies suggest the sensitivity of the modified method in detecting ChE inhibition caused by organophosphates and possibly carbamates [14,20,23-25]

Measurement of blood ChE activity in people is a non-invasive biomarker method for monitoring poisoning or exposure to organophosphate and carbamate insecticides [21,26, 27]. These results further support and expand previous findings, and the modified method was validated for determining ChE activities in the blood of people [17,20]. Furthermore, the organophosphate and carbamate insecticides decreased Che activities in several animal species [23- 25, 28].

In conclusion, the present study extends the usefulness of the described electrometric method by detecting ChE inhibition in workers exposed to insecticides

Acknowledgements

This study was supported by the Technical College, Kirkuk, Iraq.

The author thanks Prof. Dr. F. K. Mohammad, University of Mosul for his support and advice.

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