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Inhibition of Lymphocyte Neuropathy Target Esterase and Erythrocyte Acetylcholinesterase in Workers Exposed to Fenthion

Fentiona Maruz Kalan İşçilerde Lenfosit Nöropati Hedef Esteraz ve Eritrosit Asetilkolinesteraz İnhibisyonu

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Abstract

Lymphocytic neuropathy target esterase (NTE) and acetylcholinesterase (AchE) levels were measured in workers exposed to fenthion in order to assess if this insecticide is a potential delayed neurotoxicant in humans. While a maximum of 35% inhibition (4.6 IU/ml pre-exposure, 3.0 IU/ml post-exposure) was observed in AchE enzyme activities in the case group post-exposure , a maximum of 16% inhibition (14.9 nmol/min mg protein pre-exposure, 12.6 nmol/min mg protein post-exposure) was assessed in NTE enzyme activities. Data accumulated from this study was discussed in the light of available data from similar animal studies using fenthion, since no similar studies were available about neuropathy target esterase levels in humans exposed to fenthion.

Key words: Fenthion, lymphocytic neuropathy target esterase, organophosphorus-induced delayed polyneuropathy

Introduction

Some organophosphorus (OP) compounds cause a toxicity known as OP induced delayed polyneuropathy (OPIDP). It is defined as a central-peripheral distal sensory-motor axonopathy. Signs of the axonal neuropathy take 2-5 weeks to appear in animals and man. This delayed peripheral polyneuropathy differs from the acute cholinergic phase clinically and pathophysiologically. The proposed mechanisms of OPIDP have been extensively

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reviewed (Lotti, 1986; Jonson, 1987; Abou-Donia and Lapadula, 1990; Johnson, 1990; Lotti, 1992; Johnson and Glynn, 1995). OPIDP is initiated by the phosphorilation of the active site of a specific enzyme in the nervous system called neuropathy target esterase or neurotoxic esterase (NTE). A second step is then required to produce the toxic effect, the "aging" of the phosphoryl-enzyme complex. NTE activity has been detected in man's peripheral lymphocytes. The measurement of enzyme activity was suggested as a biological test to monitor lymphocyte NTE in workers exposed to a potentially neurotoxic OP (Bertoncin *et al.*, 1985).

Fenthion (o,o-dimethyl o-4-methyl-thio-m-tolyl phosphorothioate) is one of the most widely used OP insecticides. Its uses include spraying for mosquito control, ground application for control of cereal bugs, leafhoppers, and rice stem larve, fumigation against fruit flies, and in veterinary practice. This insecticide can be qualified as a potent acute organophosphorus neurotoxicant (LD_{50}: 30-40 $mg.kg^{-1}$ in chick and hen) (Cherniack, 1988.) However, there is little information about its delayed neurotoxic effects in man (Metcalf *et al., 1985;* Karademir *et al., 1990.*) On the other hand, organophosphate compounds including fenthion are frequently used for agricultural purposes in Turkey.
This study has therefore been undertaken to evaluate the inhibitory effect of one day single dose fenthion exposure on the lymphocyte NTE and erythrocyte acetylcholinesterase (AChE) activities of workers.

Materials and Methods

Blood samples were obtained from four municipal workers who had not been exposed to any kind of insecticide for one month but sprayed Lebaycid 50 EM (52.5% fenthion) to the public gardens and the green houses in Ankara prior to sample taking and one worker who stocks this insecticide. These five workers were subjected to detailed history taking and noting their working conditions. Venous blood was collected to heparinized tubes three times from each worker as; prior to, 24 h and two months after spraying.
Lymphocytes were isolated by centrifugation on histopaque within 6h (Boyum, 1968). Lymphocyte NTE activity and protein determination were assayed according to Johnson (1977) and Lowry *et al.* (1951) respectively. Pellets obtained after washing and decanting the supernatant were stored at -80^0 C until assayed. Erythrocyte AChE was assayed as described by Ellman *et al.* (1961).
Differences between values were evaluated by Wilcoxon signed rank test.

Results

The general characteristics of workers with regard to age, sex, the amount of insecticide sprayed, the area sprayed and the duration of exposure are summarized in table 1.

AChE and lymphocyte NTE levels of workers before and 24 h and 2 months after exposure are shown in tables 2 and 3.

Table 1 - The general characteristics of workers

Subjects	Sex	Age	Lebaycid 50 EM (ml)	Area (m^2)	Duration of exposure (hrs)
1	M	39	300	500*	3
2	M	33	300	500*	3
3	M	30	600	10000**	6
4	M	27	Stocking-Worker	-	-
5	M	31	600	10000**	6

*Green house , **Public garden

Table 2- AchE levels of workers exposed to fenthion.

	AchE Activities (IU ml^{-1})			
Subjects	Before exposure	After exposure (24 h)	% Inhibition	Two months later
1	3.1	3.3	-6.4	3.1
2	3.2	2.9	10.0	3.2
3	4.6	3.0	35.0	2.6
4	3.1	3.7	-19.3	2.8
5	4.7	4.1	13.0	3.9
Mean	3.7±0.8	3.4±0.5		3.1±0.5

*The difference between before and after exposure NTE was marginally significant (p=0.0625).

Table 3- Lymphocyte NTE levels of workers exposed to fenthion.

	NTE Activities (nmol/min.mg protein)			
Subjects	Before exposure	After exposure (24 h)	% Inhibition	Two months later
1	12.2	11.2	3.0	12.5
2	14.6	12.7	13.0	14.7
3	8.2	7.0	15.0	8.6
4	14.0	14.6	-4.3	14.3
5	14.9	12.6	16.0	13.8
Mean	12.8±2.7	11.6±2.8*		12.8±2.4

*The difference between before and after exposure NTE was marginally significant (p=0.0625).

Discussion

Data accumulated from this study had to be discussed in the light of available data from similar animal studies using fenthion since no similar studies were available about NTE levels in humans exposed to this insecticide. However, animal studies on correlation between NTE inhibition in brain and lymphocytes and the development of OPIDP are in conclusive and have not been yet completely validated in humans. There is little evidence about the delayed neurotoxic effects of fenthion in man. In a veterinary hospital, three persons who handled fenthion during animal dipping displayed signs of OPIDP (Metcalf *et al.*, 1985). There was also a reported suicide attempt with fenthion developed delayed neuropathy in our country (Karademir *et al.*, 1990). NTE was not measured in any of these cases. This picture was complicated by some reports in hens. Gaines (1969) and Francis *et al.* (1985) described a syndrome of prolonged leg weakness which lasted from 10-50 days in hens exposed to fenthion. According to the results of Farage-Elavar and Francis (1987,1988), although fenthion given either orally or dermally in single or multiple doses to young chicks did not significantly inhibit NTE, it caused prolonged effects on gait including decreased length and increased width of stride and fenthion treated chicks developed an atypical ataxia at the normal age for onset of sensitivity to OPIDP. On the other hand, although fenthion did not inhibit either the lymphocyte or brain NTE significantly, AChE inhibition reached to 66% 24 h after single oral dose of 5 $mg.kg^{-1}$ to young chicks. Many factors could account for these discrepancies, including dose ,duration of exposure, administration route and genetic differences, so the extrapolation of these findings to man should be done very carefully. In our study, we observed that there was a statistically significant correlation between the percent inhibitions of AChE and lymphocyte NTE activities ($r = 0.8929$, $p<0.05$). After exposure, while a maximum of 35% inhibition (4.6 $IU.ml^{-1}$ pre-exposure and 3.0 $IU.ml^{-1}$ post-exposure) was observed in AChE activities of workers, a maximum of 16% inhibition (14.9 nmol/min.mg protein pre-exposure, 12.6 nmol/min.mg protein post-exposure) was assessed in NTE activities. Two months later, while AChE inhibition persisted, NTE levels rose to the normal value.

In conclusion, although available data from animal studies of short term exposure to fenthion were not sufficient to make a proper judgement of risk, due to the differences in dose, duration of exposure and species, our findings showed that fenthion inhibited NTE activity as much as AchE. However, the sample size in this study was too small to draw a conclusion and further studies with larger size samples are required for a better definition of the nature and mechanism of the effect. On the other hand, in many developing countries, OP insecticides are heavily used in agriculture and are even present at homes and fenthion is one of these insecticides. Chronic exposure, poor education and working conditions result in serious occupational intoxications. Cholinesterase inhibition is used as an indicator to monitor OP exposure. When the level of NTE inhibition without any toxic effect is established, the measurement of lymphocytic NTE might be used as a clinical test to predict and prevent the development of OPIDP.

Özet

Fentiona maruz kalan işçilerde, bu insektisitin insanlarda potansiyel gecikmiş etkili bir nörotoksikan olup olmadığını belirlemek amacıyla lenfositik nöropati hedef esteraz (NTE) ve asetil kolin esteraz (AchE) seviyeleri ölçülmüştür. Maruziyetten sonra AchE enzim aktivitesinde maksimum % 35 inhibisyon(4.6 $IU.ml^{-1}$ maruziyet öncesi ve 3.0 $IU.ml^{-1}$ maruziyet sonrası) gözlenirken, NTE enzim aktivitesinde ise %16' lık bir inhibisyon (14.9 nmol/dk mg protein maruziyet öncesi ve 12,6 nmol / dk mg protein maruziyet sonrası) belirlenmiştir. Fentiona maruz kalan insanlardaki NTE seviyeleri ile ilgili benzer çalışmalar olmadığından, ancak benzer hayvan çalışmalarından elde edilen sonuçlar ile bu çalışmada bulunan veriler karşılaştırılarak tartışılmıştır.

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