Indian J Physiol Pharmacol 1996; 40(3): 249-252

CORRELATION BETWEEN THE SEVERITY OF SYMPTOMS IN ORGANOPHOSPHORUS POISONING AND CHOLINESTERASE ACTIVITY (RBC AND PLASMA) IN HUMANS

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(Received on September 20, 1995)

Abstract : This study was undertaken to examine the correlation, if any, between the inhibition of red blood cell cholinesterase (RBC ChE), plasma cholinesterase (PChE) and cerebrospinal fluid acetyl cholinesterase (CSF AChE) and the severity of symptoms in patients poisoned with organophosphorus (OP) compounds. Baseline values of the cholinesterases (RBC, Plasma & CSF) were established in our laboratory using a modified colorimetric method. OP poisoned patients were divided into 3 groups – mild, moderate and severe based on clinical symptoms. We observed a severity dependent inhibition of both RBC ChE and PChE, in acute poisoning. Sequential post exposure estimations of the ChEs upto 5 days did not reveal any rise in the values though there was substantial clinical improvement. Our findings therefore indicate that the correlation of ChE values with severity of symptoms are applicable only in the initial stages of acute poisoning. AChE could not be detected in CSF in two severely neurotoxic patients who subsequently expired. The clinical significance of this observation needs to be examined further.

Key words : organophosphorus poisoning cholinesterases

INTRODUCTION

In acute organophosphorus (OP) poisoning, both plasma cholinesterase (PChE) and red blood cell cholinesterase (RBE ChE) activities are markedly decreased below the base level. Since RBC ChE activity more closely reflects acetylcholinesterase (AChE) activity in the nervous tissue (1, 2), depression of RBC ChE when used alone is a confirmatory test for OP poisoning. Further, depression of RBC ChE is more consistent and may persist for as long as 12 weeks while PChE depression may only last for 1-3 weeks and is a poor indicator of toxicity. In neurotoxic patients CSF AChE values may be of use for prognostic purposes. As a routine in clinical practice only PChE is estimated in cases of OP poisoning though for reasons mentioned above RBC ChE or CSF AChE may be a better index of the severity of the poisoning.

The present study was undertaken (a) To evaluate the extent of depression of RBC ChE and PChE in OP poisoned cases and to see the correlation if any with changes in these enzymes to the severity of the poisoning with a view to utilise them for predictive and prognostic purposes, (b) To evaluate changes if any in CSF AChE in severely and neurologically toxic patients.

METHODS

Thirtyseven patients of either sex and any

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age admitted to St. John's Medical College Hospital within 2-4 hr of having ingested an OP compound and *not having been treated* outside were included in the study. Approval of Ethical Committee of our Institution was obtained. Of the 37 patients, 30 patients had consumed Folidol (Ethyl parathion) and 7 patients Tik 20 (Sumithion). Quantity consumed varied from 25 ml to 50 ml.

Severity of poisoning based on clinical symptoms was classified into mild, moderate and severe (3).

Mild: Fatigue, headache, dizziness, numbness of extremities, nausea, vomiting, excessive sweating, salivation, tightness in chest, abdominal cramps and diarrhoea.

Moderate: Inability to walk, generalised weakness, difficulty in talking, muscular fasciculations, miosis and increase in severity of symptoms described under mild poisoning.

Severe: Unconsciousness, not responding to painful stimuli, marked miosis, loss of pupillary reflexes to light, flaccid paralysis, nasal and salivary secretions, crepitations in the lungs, respiratory difficulty and cyanosis.

On admission and prior to any treatment 3 to 4 ml of venous blood was collected in glass tubes containing 0.5 ml of 10% di-sodium ethylene diamine tetra acetic acid (EDTA). The samples were centrifuged at 3000 rpm for 15 min and the plasma and RBC separated. RBC ChE and PChE were estimated within 24 hours of sample collection. In 5 patients blood samples were also collected for RBC ChE and PChE estimation on the 2nd and 5th days, post-treatment. Treatment consisted of the following : Stomach wash on admission, intravenous drip with 5% dextrose-normal-saline; Atropine 0.6 mg i.v. every 15 mins (Atropine was discontinued once signs of toxicity were observed, viz. pulse $\geq$ 110 fixed pupils non reactive to light, temperature $\geq$ 102°F restlessness in the patient. Pralidoxime 2 g i.v. Q8h for 48 hrs. Crystalline Penicillin 10 Lacs i.v. Q6h in patients who had aspirated.

CSF (0.2 - 0.5 ml) was collected and analysed for CSF AChE in 2 patients with severe neurological symptoms.

Baseline values for RBC ChE and PChE's (n = 19) were obtained from blood samples obtained from control subjects consisting of (a) Healthy blood donors of either sex and various age groups who underwent a complete screening procedure for haemoglobin, liver function test, hepatitis B antigen, sexually transmitted diseases and human immunodeficiency virus, (b) Medical students and staff of either sex and various age groups who were considered normal by routine physical examination and laboratory tests.

Baseline values for AchE in CSF (n = 10) 0.2 to 0.5 ml of CSF was collected and estimated for AchE from otherwise healthy patients undergoing short surgical procedures like transurethral resection of prostate, excision of encysted hydrocoele etc. under spinal anaesthsia.

Analytical procedure: Modified colorimetric method of Ellman et al (4) as described by Venkataraman et al (5) was used for the estimation of enzyme activities. Acetylcholine iodide and buterylthiocholine iodide were used as substrates for RBC/CSF AchE and PChE respectively. The activities were expressed in units wherein 1 unit of activity was equivalent to 1 µmol of thiocholine liberated in 1 min/1 g of haemoglobin or 1 min/1 ml of plasma or CSF (5).

Statistical analysis: The inhibition of RBC ChE and PChE & CSF AChE produced by OP compounds in mild, moderate and severe poisoning was compared to baseline values in controls using the Students 't' test.

RESULTS AND DISCUSSION

Of the thirtyseven patients, 14 were classified as mild poisoning, 12 as moderate poisoning and 11 as severe, based on clinical symptoms (3). We have compared post-exposure values of the ChE's of the OP poisoned cases with the normal values set in our own laboratory. As each laboratory has a different set of normal

values and reporting units, we are unable to compare our laboratory ranges with those reported earlier due to methodological differences and differences in expression (6-8).

The mean ± SE of RBC ChE and PChE levels in healthy volunteers as well as in the OP poisoned cases in this study are shown in Table I. The % inhibitions of the ChE in all three categories of poisoning is also indicated in the Table I. A severity dependent inhibition of both RBC ChE and PChE was seen in the OP poisoned patients ($P < 0.001$). The inhibition of of ChE depression but should act on his clinical impression, experience and history of exposure, because even with RBC ChE activity reduced by 25% 46-90% of the population would still have activity above the lower limit of normal, and PChE activity 25% inhibited 92-99% would still have activity within the normal range (10). If the clinician is not aware of this a false-negative diagnosis can be made.

In moderate to severe poisoning the clinical signs and symptoms are fairly clear and verification by laboratory tests is also quite

TABLE I : RBC ChE and PChE values (Units) in healthy volunteers and in mild, moderate and severe organophosphorus poisoned patients.

Group	*(n)*	*RBC ChE*	*% Inhibition*	*PChE*	*% Inhibition*
Healthy volunteers	(19)	102.16 ± 5.26	–	7.07 ± 0.22	–
Mild poisoning	(14)	67.50 ± 5.50*	34	4.88 ± 0.26*	31
Moderate poisoning	(12)	19.16 ± 4.58*	81	2.85 ± 0.33*	60
Severe	(11)	8.36 ± 3.78*	92	1.09 ± 0.44*	84

*P <0.001 compared to control (healthy volunteers).

n in parenthesis denotes number of subjects.

RBC ChE being 33.93%, 81.25% and 91.82% in mild, moderate and severe poisoning. The corresponding inhibition for PChE was somewhat similar being 30.98%, 59.69% and 85.58% respectively. This is in agreement with earlier observations in acute poisoning wherein it has been reported that PChE activity is 20-50% of normal in mild poisoning, 10-20% of normal in moderate poisoning and less than 10% in severe poisoning (2, 3).

It has been stated that OP poisoning may be diagnosed only when PChE or RBC ChE falls below 50% of normal (9) although authoritative texts state that patients suffering from mild poisoning may exhibit ChE values within the laboratory range (10). The range of symptoms and degree of ChE inhibition observed in mild OP poisoned patients in our study indicate that mild poisoning can occur with less than 50% ChE inhibition. Therefore in mild posioning with single toxic exposure the clinician should not depend entirely on laboratory values dependable, wherein a substantial inhibition of both enzymes occur.

Though it has been reported that the OP compounds depress both the enzymes, only depression of RBC ChE is a specific response to these toxins (2, 9), in our study we observed no significant difference in the degree of inhibition of RBC AChE and PChE.

We are therefore of the opinion that in acute poisoning PChE can be taken as a good indicator of the severity of OP poisoning.

Sequential post-exposure ChE determination in 5 OP poisoned cases did not show any improvement in RBC ChE or PChE levels upto 5 days postexposure. On admission the values for RBC ChE and PChE were 40.78 ± 9.99 and 3.12 ± 0.73 units respectively. The corresponding values after 48 hr were 41.58 ± 10.3 and 3.67 ± 0.64 units and on 5th day 41.58 ± 10.2 and 3.57 ± 0.64 units. It may be noted that the significant inhibition of both RBC ChE and

PChE ($P < 0.001$) noted on admission continued for all 5 days though the patients improved clinically with the standard treatment consisting of atropine, pralidoxime and intravenous fluids. This is not unexpected as RBC ChE regenerates only at approximately 1% per day and PChE at approximately 25% in the first 7 to 10 days (9). Our findings also confirm that the correlation between the degree of ChE inhibition and severity of manifestations is pertinent only in the initial stage of acute poisoning (3). Thus the warning that an individual who has been acutely poisoned showed inhibition of ChE activities should not be allowed to return to work with OP compounds until the ChEs return to approximately 75% of normal (10) should not be ignored.

Neurological symptoms are a prominent feature of OP poisoning particularly in severely poisoned cases. Since CSF may provide a biochemical 'window' into the brain and yield useful indices (11) we felt that assay of CSF for AChE and PChE might give useful clues in differential diagnosis particularly because CSF AChE activity and total protein vary independently (12).

In our laboratory levels of CSF AChE in normal individuals were 0.44 ± 0.03 units much less than that found in RBCs. PChE could not be detected in CSF. Reported values in literature are 17.5 ± 4.1 and 21.5 ± 5.6 nmols/min/ml CSF for AChE and 11.3 ± 2.9 and 19.7 ± 2.8 nmol/min/ml CSF for PChE respectively (12, 13).

In the two cases of severe poisoning wherein we measured the AChE activity the values were zero indicating that a complete inhibition of the enzyme by the OP compounds. These two patients were severely toxic and proved to be fatal. The clinical significance and usefulness of CSF AChE assay in OP poisoning needs to be evaluated since it may be of prognostic value in severely neurological toxicity.

ACKNOWLEDGEMENTS

Financial support from the Karnataka State Council for Science and Technology is gratefully acknowledged.

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