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Correlation between Cholinesterase and Paraoxonase 1 Activities: Case Series of Pesticide Poisoning Subjects

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ABSTRACT

Introduction: Acute exposure to pesticide due to suicidal poisoning is the most extensive cause of pesticide exposure, compared with all other causes including agricultural or industrial exposure. Organophosphate (OP) and carbamate group of pesticides can inhibit acetylcholinesterase; on the other hand, paraoxonase1 can detoxify organophosphate poisoning by hydrolyzing organophosphate metabolites. ***Methods:*** We have compared the serum paraoxonase1 status and cholinesterase activity of subjects who attempted to commit suicide by consuming OP pesticide. Cholinesterase and paraoxonase1 activity were measured spectrophotometrically using butyrylthiocholine and phenyl acetate as substrates, respectively. ***Results:*** A positive correlation was found between serum paraoxonase1 activity and cholinesterase activity among pesticide consumed subjects. ***Conclusion:*** Our results suggest that subjects with higher paraoxonase1 activity may have a better chance of detoxifying the lethal effect of acute organophosphate poisoning.

Introduction

Pesticides and insecticides are synthetic chemicals used worldwide in controlling agricultural as well as domestic pests. Consequent to their indiscriminate use, humans are exposed to chronic and sometimes to acute exposures to these toxic chemicals. The chronic exposure is mainly through pesticide residues in fruits and vegetables. A recent study on random samples of vegetables and fruits showed that 11.5% of vegetables and 7% of fruits were contaminated with pesticide residues.[1] Hence, not only the pesticide manufacturers and agricultural workers are exposed to pesticides, but even the general public are at a risk of chronic exposure to pesticides.

Acute exposure to pesticide occurs frequently through accidental exposure in few cases, but in most cases, it occurs due to consumption of pesticides during suicide attempts. According to World Health Organization, about 17,000 deaths in India occur by suicide every year.[2] About 50% of suicidal deaths are due to consumption of pesticides. In India, mainly four types of pesticides are used, namely: organophosphates, carbamates, organochlorines and pyrethroid pesticides. The mode of action of organophosphorus (OP) and carbamate pesticides is through inhibition of Acetylcholinesterase (AChE) activity. In fact, measurement of cholinesterase activity is used as a diagnostic and prognostic tool in pesticide poisoning.[3]

An enzyme which could detoxify OP compounds in animal tissues was reported in 1946 and in human serum in 1953.[4,5] The enzyme was called 'A' esterase and subsequently, it was called paraoxonase1 (PON1). Paraoxonase activity of the enzyme PON1 is its promiscuous activity, whereas the main activity of the enzyme is lactonase activity.[6] Among promiscuous substrates, this enzyme can detoxify parathion, diazinon, chlorpyrifos and the nerve agents sarin and soman.[7] PON1 activity of an individual can vary to a large extent. It is reported that PON1 levels can vary by at least 13-fold and the activity up to 40-fold.[8] This implies that the ability of individuals to detoxify OP would depend on their PON1 activity. In this study, we have compared the PON1 activity and cholinesterase activity of subjects who attempted to commit suicide by consuming pesticides.

Materials and methods

Materials

Phenylacetate, $CaCl_2$, and Tris were purchased from Sigma Aldrich Co. (St. Louis, MO, USA). Liqui Cholinesterase kit (catalog no. 2105) was from Futura Systems (Rome, Italy). All other reagents used were of analytical grade and solvents were distilled before use.

Methods

Subjects

Study group consisted of 58 pesticide poisoned cases

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who were admitted to K R hospital Mysore, India, from August 2009 to January 2010. They were all the cases admitted for acute poisoning with pesticides, and formed the 'convenience sample'. The period of the study was arbitrarily chosen to represent half a year. Among them, 43 subjects were males and 15 subjects were females (age group from 14 to 70 years old; mean 30.26 ± 12.36). They were diagnosed as OP poisoning by an emergency room physician using clinical signs and symptoms. Blood serums of these subjects were drawn immediately after admitting and were used for enzyme assays or kept frozen at –20°C for future use. All the experiments involving human subjects were carried out in accordance with the protocol approved by Institutional Human Ethical Committee, University of Mysore, Mysore [Sanction order no. IHEC-UOM No. 38/PhD/2009-10], India.

PON1 status

The level of PON1 activity was determined by measuring arylesterase (AREase) activity in serum using phenyl acetate as a substrate. A 10µl of diluted serum (1:10 v/v) was added to 10mM Tris-HCl buffer, pH-8.0 containing 2mM $CaCl_2$ and 2mM phenyl acetate. The rate of generation of phenol was determined at 270 nm at 25°C, using a continuously recording spectrophotometer.[9] One enzyme unit was defined as the amount of enzyme that catalyzes the hydrolysis of 1µmol of substrate per minute.

Cholinesterase measurement

Cholinesterase activity of serum was determined using Futura Systems Liqui Cholinesterase kit (catalog no. 2105), which is based on a colorimetric assay technique. Thiocholine, released from butyrylcholine, reacts with Hexacyanoferrate III (yellow) to form Hexacyanoferrate II (clear). The decrease in absorbance was determined photometrically at 405 nm, and is directly proportional to Cholinesterase activity in the serum. One unit of enzyme catalyzes the production of 1µmole of thiocholine per minute under the assay conditions (pH-7.7 and 37°C) (normal values 4200-11200 U/L).

Statistical analysis

Correlation between PON1 and cholinesterase was made using Pearson product moment correlation coefficient. Difference between groups was tested using student's t -test at $p \leq 0.05$.

Results

PON1 activity and cholinesterase activity were determined in serum of the subjects who had consumed pesticides in an attempt to commit suicide. The findings of enzyme levels are summarized in Table 1. There was a significant difference in cholinesterase activity between the groups with low PON1 activity and high PON1 activity. Serum PON1 activity was positively correlated with cholinesterase activity (r = 0.2843; critical value for 56 df = 0.258) in suicidal subjects (Fig. 1). Fig. 2 shows the cholinesterase level among low PON1 activity group and high PON1 activity group. Relative to the high serum PON1 group (group 2), low serum PON1 group (group 1) had higher inhibition of cholinesterase, and the results were significant ($p \leq 0.05$).

Discussion

Cholinesterase, also known as plasma cholinesterase or pseudocholinesterase or butyrylcholinesterase, belongs to the same structural class of proteins as AChE.[10] AChE is a serine protease that hydrolyzes neurotransmitter acetylcholine at the neuromuscular junctions and cholinergic brain synapses, where its activity serves to terminate synaptic transmission. AChE, primarily found in the neural synapses and also on the surface of erythrocytes, maintains the integrity of erythrocytes.[11] Cholinesterase is synthesized in liver and secreted into plasma. It preferentially acts on butyrylcholine and hydrolyzes acetylcholine.[12] Cholinesterase activity can be measured in serum as surrogates for neuronal AChE activity. Cholinesterase measurement is used as diagnostic tool for pesticide poisoning because of advantages such as simple detection procedure, stable nature, easy-to-sample and reproducibility.[13] Each person has a certain normal basal level of activity for the proper functioning of the nervous system. Variations in cholinesterase activity in blood are observed in various clinical conditions including the entry of natural (snake venom) or synthetic toxins into the human serum.[14,15]

Inhibition of AChE activity in the central and peripheral

Table 1. Mean activities of serum PON 1 and cholinesterase in experimental groups

Subjects	(n)		Activity	
			PON1 [AREase activity (U/l)]	Cholinesterase activity (U/l)
Total subjects involved in the study	58	PON1 activity of all the subjects	77759 ± 3482 Median=79389	3474.1 ± 330.48 Median=2961
Group1	16	Subjects with low PON1 activity (Lowest 1/3rd)	43893 ± 2922 Median= 44275	2528 ± 433.82 Median = 2102
Group2	18	Subjects with high PON1 activity (Highest 1/3rd)	108283 ± 3340 Median=104326	4576 ± 745.53 Median=3927.5

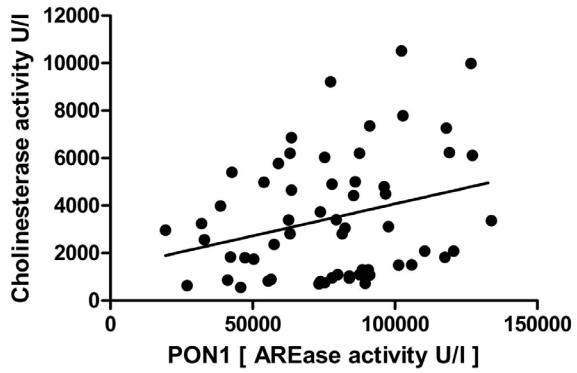

Fig. 1. Correlation between PON1 and cholinesterase activities found using Pearson product moment correlation coefficient (r = 0.2843, Critical value of 56 df = 0.258).

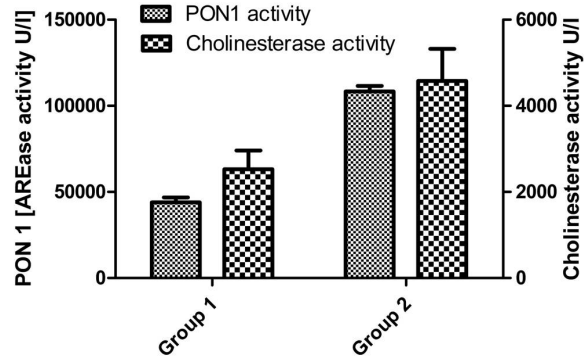

Fig. 2. Cholinesterase level among PON1 activity groups [Group1: low PON1 activity; Group2: high PON1 activity]. Results are significant ($p \leq 0.05$) when compound with corresponding values of group 1.

nervous systems is considered to be an important mechanism of organophosphate toxicity. AChE inhibition prevents the breakdown of acetylcholine, resulting in excessively increased cholinergic activity at the nerve synaptic gaps.[16] Excess accumulation of acetylcholine at muscarinic receptors causes clinical complication such as visual disturbance, tightness in chest, wheezing due to broncho-constriction, increased bronchial secretion, increased salivation, lacrimation, sweating, peristalsis and urination.[17,18]

OPs are bioactivated *in vivo* via oxidative desulfuration and dealkylation to form its oxygen analogues (Oxon's) and other active metabolites.[19] These OP-oxygen analogues are potent inhibitors of the enzyme AChE. Serum PON1 can hydrolyze the oxygen analogs of OP and it is very important in OP detoxification process.[20] The substrate specificity of PON1 is unusually broad and not fully understood.[21] AREase activity is considered to be a good surrogate for PON1 concentration in plasma/serum.[22,23]

In our study, PON1 activity was significantly correlated with levels of cholinesterase in subjects who consumed pesticides. The individuals with lower PON1 activity (group 1) also had lower cholinesterase activity, suggesting the inhibition of cholinesterase by pesticides. In contrast, individuals with higher PON1 activity (group 2) had higher cholinesterase activity, indicating the involvement of PON1 in detoxification of pesticide poisoning (Fig. 2). The findings of our study indicated the association between low plasma PON1 activity and cholinesterase inhibition and are consistent with the results of Sozmen *et al.* and Hofmann *et al*.[24,25] They found that PON1 activity was lower among subjects with low cholinesterase activity upon hospital admission relative to subjects with higher cholinesterase activity. Studies in transgenic mice (e.g. Li *et al.*) clearly demonstrated that low plasma PON1 activity was associated with greater brain AChE inhibition after exposure to chlorpyrifos oxon and diazoxon.[26] They also found that administration of PON1 abolished cholinergic signs and significantly protected it against AChE inhibition.[27] Supporting our data, Akgur *et al.* in 2000 also found positive correlation between AChE and PON1 activities in a study with 18 agricultural male workers who were exposed to OP poisoning in Turkey.[28]

Limitations

Although the study has achieved its aims, there was a limitation. It was not possible to follow up the outcome of the treatment of suicide subjects since some of them were shifted to private clinics. Hence, the survival or otherwise of the subjects was not known.

Conclusion

PON1 and cholinesterase in the serum of subjects with pesticide poisoning were measured and found to correlate positively. Our study suggests that patients with higher paraoxonase1 activity may have a better chance of detoxifying the poisoning effects of pesticides and may have a positive effect on survival even though this could not be verified by this study.

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Ethical issues

All the experiments involving human subjects were carried out in accordance with the protocol approved by Institutional Human Ethical Committee, University of Mysore, Mysore [Sanction order no. IHEC-UOM No. 38/PhD/2009-10], India.

Competing interests

The authors declare that they have no competing interests.

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