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Human Acetyl cholinesterase Inhibition by in vivo and in vitro Pesticide Exposure

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ABSTRACT

The purpose of this review is a comparative study of different types of anticholinesterases (pesticides, heavy metals and drugs etc.) and their actions based on biochemical estimation of AChE activities in human tissues. The study contains the presentation of inhibitory action of organophosphates, carbamates, organochlorines and drugs on human AChE activities in vivo as well in vitro. Suicidal cases (via ingestion), accidental exposure (via respiratory tract), occasional exposure to pesticides at their working place, pathological conditions and in vitro experiments have been studied and discussed to know the level of inhibition of human AChE due to varied concentrations of toxicants. Besides, behavioral symptoms expressing the extent of AChE inhibition, the treatment procedure and follow-up reports from hospital during treatment of pesticidal toxicity have also been discussed.

Keywords: Pesticide poisoning, human Ach inhibition

Introduction

The persistence of the organophosphorus pesticides in the environment is very harmful for human health because they irreversibly inhibit the catalytic active sites of acetylcholinesterase (AChE) - enzyme essential for the functioning of the central nervous system in human and insects,[1,2] which catalyses the hydrolysis of the neurotransmitter - acetylcholine (ACh). As result the accumulation of the ACh, may interferes with muscular responses[2] and may induce symptoms ranging from increased salivation and headache, to convulsion and suppressed breathing which can produce eventually death.[3] Also, pesticides can be carcinogenic, citogenic, they can produce bone marrow diseases, infertility, nerve disorders, immunological or respiratory diseases and organophosphate-induced delayed polyneuropathy.[4]

Carbamate pesticides are preferred because of their high specificity to target organisms and their low bioaccumulation in lipids and body tissues in comparison to the chlorinated hydrocarbon pesticides.[5] In recent years, growing attention has been paid to the development of reliable, fast and inexpensive analytical system able to monitoring pesticides from agriculture, environmental and food industry production.

(I) Pathological conditions and AChE inhibition

During pathological conditions AChE has been proved to be a remarkably significant as a diagnostic tool. Mickelson et al examined the content and distribution of skeletal muscle disorder malignant hyperthermia (MH).[6] The AChE activity of sarcolemma membranes isolated from MH- susceptible (MHS) swine was increased two fold when compared with normal sarcolemma. The total AChE activity of muscle extracts was also doubled in MHS tissue, however, the relative distribution between lo-salt extractable (globular forms) and high-salt extractable (asymmetric forms) AChE activities were similar in MHS and normal muscle.

(II) Pesticide exposure and AChE inhibition

a) Skin exposure

The approach of setting an occupational exposure limit for phosphamidon, an organophosphorus pesticide in the workplace in China has been described.[7] They suggested a maximum allowable concentration (MAC) for phosphamidon in the air of the workplace as 0.02 mg/m³ but on the condition that the route of skin entry be blocked effectively. In other study, a large number of workers in agriculture (through skin contact) were exposed to pesticides either directly during mixing and loading or indirectly due to contact.[8] They investigated the influence of skin moisture on the dermal uptake of the pesticide propoxur. The study was conducted in human volunteers under controlled temperature conditions (30°C) and environmental relative humidities of either 50, 70 or 90%. The study was approved by the Medical Ethics Committee. In their study a linear relationship between the environmental relative humidity and the level of skin moisture was observed. The results indicated that the level of skin moisture influences the absorption of propoxur via the dermal route, dramatically ranging from, on average, 13, 33-63% of the potentially absorbed dose' which is excreted in urine as the primary metabolite 2-isopropoxyphenol (IPP) at relative humidity levels of, on average 50, 70 and 90%, respectively. The 'potentially absorbed dose' is defined as the difference between the applied dose and the dislodged dose after 4 h. They concluded that by assessing health risks of workers in agriculture exposed dermally to pesticides and e.g. in testing the efficiency of protective clothing under realistic conditions, the influence of the level of skin moisture on absorption of substances may be considerable and has to be taken into account.

b) Respiratory exposure

Respiratory exposure of pesticides has long been known for causing, anticholinesterase action, behavioral symptoms, stress etc. Cases of aluminium phosphide do occur in northern India, whereas, in western India the organochlorine compound endosulfan, the organophosphorus compounds dimethoate, thimet (phorate) monocrotophos, fenthion, dimecron (phosphamidon) nuvan (dichlorvos), malathion, fenitrothion, methyl parathion and metasystox (demeton-s-methyl) and the carbamate baygon (dichlorvos + propoxur) account for far more deaths.[9,10] Clinical,

hematological and biochemical studies were conducted on 34 subjects, who were employed in spraying mango trees with monocrotophos, phosphamidon, dichlorvos, oxydemeton-methyl, malathion, endosulfan, parathion-methyl, dimethoate or carbaryl throughout the year.[9] Findings were compared with those of 14 control subjects. Inhibition of ChE activity was observed in the exposed group.

Consequently, acting these inhibitors inhaled through respiration, their products effectively impair restoration of proper cholinergic levels and hence contribute to impairment of the dopaminergic-cholinergic balance. A case report of an urban family was studied, who suffered excessive exposure to organophosphorus and carbamate pesticides (dichlorvos and propoxur) applied as bombs pressurized canisters for the control of an infestation of fleas, ctenocephalids in their apartment.[11] All three family members developed symptoms compatible with ChE inhibition, such as headache, lightheadedness, wheezing, shortness of breath, nausea and fatigue. Serial measurement of red blood cell and serum ChE soon after exposure and during subsequent months confirmed the diagnosis of pesticide poisoning. Data on cholinesterase test results of 73 municipal and 65 mosquito abatement district (MAD) workers gathered in 1985 was examined.[12] Overall, none of the pesticide applicators would have been removed from exposure to cholinesterase inhibiting compounds by the existing threshold criteria established for agricultural pesticide applicators.

c) Ingestion

Behavioral disorders

The increased widespread application of organophosphate pesticides has under effect on human behavior. Clinical reports and laboratory investigations have generally supported the assumption that neurobehavioral manifestations of organophosphate toxicity are attributable to accumulation of acetylcholine at central and peripheral synapses as a result of cholinesterase inhibition.[13] Investigators generally agree on the presence of several behavioral sequelae of organophosphate poisoning: impaired vigilance and reduced concentration, slowing of information processing and psychomotor speed, memory deficit, linguistic disturbance, depression and anxiety and irritability. Studies of asymptomatic workers was undertaken at risk for repeated exposure to organophosphate pesticides, which produced only equivocal findings concerning the presence of less severe or latent forms of these behavioral abnormalities.[13]

A case report of a 2.5 year old boy having severe central nervous system (CNS) depression after ingestion of 500mg of chlorpromazine has been reported, followed by results of his treatment with an anticholinesterase (physostigmine).[14] Intermediate syndrome in 2 cases of organophosphorus poisoning occurred in suicidal attempts.[15] Trichorfon, propoxur, a carbamate pesticide and fenthion, a organophosphate were ingested in both attempts at suicide. After successful conventional therapy during the cholinergic phase, but before the time when the onset of delayed neuropathy might be expected, an intermediate syndrome developed. It affected the proximal limb muscles, neck flexors and respiratory muscles two days after pesticide ingestion, and both patients needed respiratory support. Recovery from the intermediate syndrome was complete in both patients although one subsequently developed delayed neuropathy.

An epidemiological study of 90 male pesticide applicators showed the effect of organophosphate pesticides mainly, guthion azinphosmethyl, phosphamidon, chlorpyrifos, phosmet and diazinon on the peripheral nervous system.[16] Acute and chronic signs and symptoms (most frequent clinical symptoms as abdominal pain, unconsciousness, convulsion, vomiting, headache and fever) of

pesticide exposure during off season and during spraying season were observed. Paired test gave that the mean vibration threshold scores for population based controls individually matched on age, sex and country of residence.

Human plasma AChE inhibition

Human plasma AChE inhibition due to organophosphorus pesticides seems to be a widely used parameter for primary evaluation of toxicity. Human subjects fed with 0.9 mg of dichlorvos (2, 2-dichlorovinyl dimethyl phosphate) 3 times a day for 21 days showed no consistent cholinomimetic signs or symptoms, nor was erythrocyte acetylcholinesterase inhibited.[17] The only significant observation was a depression of plasma ChE activity. The magnitude of the depression in plasma ChE depended on the method by which dichlorvos was incorporated into the diet. Dichlorvos, formulated as a gelatin salad and consumed during the course of the meal, was only 64% as effective in inhibiting ChE activity as when administered as a premeal capsule containing cottonseed oil. The recovery of ChE activity after dosing was terminated, which was due to the replacement of enzyme molecules. The half-life for this regeneration period was 13.7 days.

Acute poisonings in nine patients due to intentional ingestion of organophosphate pesticides has been reported.[18] Six of victims died, of which three were found dead by relatives. Six patients were treated in the Intensive care unit (ICU) of hospital from 34 hour to 45 days. Two of the patients treated in the ICU fully recovered after 25 and 24 days, while the third survivor developed delayed neuropathy. Significant fluctuations of the plasma ChE activity were observed during therapy. Postmortem revealed higher levels of pesticides in organs (e.g. 23.1 µg mention/g kidney) and in fat (135.2 µg fenthion/ g) than in the blood (4.8 µg methidathion /g) and vitreous humor.

The inhibitory actions of organophosphate pesticides on cholinesterase and in human plasma and acetone powder of human plasma has been compared.[19] Human plasma cholinesterase was inhibited at concentrations of dimethyl dichloro vinyl phosphate (DDVP) ranging from 1 X 10^{-7} M to 1 X 10^{-5} M in vitro. In particular, the cholinesterase was completely inhibited by the addition of 1 X 10^{-5} M DDVP. Cholinesterase activity in acetone powder prepared from human plasma preincubated with 1 X 10^{-3} M DDVP exhibited approximately 8-15% of that in acetone powder prepared from human plasma preincubated without DDVP. The decreased cholinesterase activities in human plasma with 1 X 10^{-7} M to 1 X 10^{-5} M DDVP and in acetone powder prepared from human plasma preincubated with 1 X 10^{-3} M DDVP were recovered by the addition of 1 X 10^{-3} M pyridine aldoxime methiodide (PAM).

Human erythrocyte AChE inhibition

Human erythrocyte measurement during pesticide exposure is also well studied. Midtling et al studied a group of 16 Hispanic cauliflower workers poisoned by the organophosphate insecticides mevinphos and phosphamidon in California.[20] They were followed in weekly clinics with interviews and determinations of plasma and erythrocyte ChE levels. Although six had initial erythrocyte ChE within the laboratory normal range, subsequent testing showed significant erythrocyte ChE inhibition. While the most severe symptoms of the 16 subjects resolved after 28 days, their erythrocyte ChE levels did not reach a plateau until an average of 66 days after exposure, after which time most patients continued to report blurred vision, headache, weakness or anorexia.

(III) Drug and AChE inhibition

The use of drugs could also inhibit AChE enzyme in human. Clozapine has been found as a new antipsychotic drug without extrapyramidal side effects and with strong sedating potency, which can produce acute symptoms of central anticholinergic toxicity.[21] Physostigmine, a reversible anticholinesterase agent which can pass the blood brain barrier, was effective in reversing the clozapine-induced brain syndrome in 2 patients. Physostigmine also reduced 1 patient's tachycardia.

(IV) In vitro toxicant exposure

In vitro studies of human blood,[22] serum,[23] erythrocyte[22 , 24 , 25 , 26] and plasma.[19] AChEs have received an attention of workers. *In vitro* studies have been performed using pralidoxime iodide up to 10 times the recommended concentrations, which produced insignificant reactivation of cholinesterases inhibited by malathion or malaoxon.[27] This was observed despite prolonged exposure of the inhibited cholinesterases to the oxime. They were of the opinion that the value of pralidoxime as a reactivator of phosphorylated cholinesterases is therefore in doubt and should not be used in preference to large doses of atropine and other supportive treatment in poisoning by organophosphate pesticides.

Serum AChE inhibition

In vitro studies on the serum ChE levels of 46 vector (*Aedes aegypti*) control workers exposed to insecticides (through residual spraying of 1g/ml of 50% fenthion e.c. and 50% propoxur e.c.) on a daily basis but without clinical manifestations of insecticide poisoning.[23] They compared ChE levels of serum of this group with those of a control group of workers who had not been exposed to insecticides at home or at work. Suppressed serum ChE levels were detected in all 46 workers exposed to insecticides, 25 of whom were aged 30-39 years. Four persons from the control group showed suppressed levels of enzyme: one of these had a genetically low level and the other 3 were on medication when the low levels were recorded.

What is already known on this topic

Organophosphate and carbamate pesticides with strong anticholinergic inhibitory action, can produce acute symptoms of central neurotoxicity. These anticholinergic agents can pass the blood-brain barrier and effectively cause AChE inhibition.

What This study adds

In this paper are reported the effects of organophosphate and carbamate pesticides *in vivo* and *in vitro* on serum cholinesterase levels of workers exposed to insecticides at work, accidentally, intake through food, water, fruit, vegetables, suicidal purposes and other cases of intoxication etc. Suppressed serum, erythrocytes cholinesterase levels were detected in all cases exposed to insecticides depending on age, sex and other factors. Behavioral symptoms that were compatible with cholinesterase inhibition are useful and give the line of treatment to be followed.

Suggestions for further development

The determination of organophosphate and carbamate pesticides in drinking water, fruit juices and vegetables should be carried out using biosensors. The biosensors consists of various types of

detectors. The estimation of K_m and V_{max} and inhibitory constants K_i in human serum exposed to pesticides would also give mechanism of inhibition of AChE.

Plasma AChE inhibition

Inhibitory actions of organophosphate pesticides on cholinesterase and in human plasma and acetone powder of human plasma have been compared.[19] Human plasma cholinesterase was inhibited at concentrations of dimethyl dichloro vinyl phosphate (DDVP) ranging from 1 X 10^{-7} M to 1 X 10^{-5} M in vitro. In particular, the cholinesterase was completely inhibited by the addition of 1 X 10^{-5} M DDVP. Cholinesterase activity in acetone powder prepared from human plasma preincubated with 1 X 10^{-3} M DDVP exhibited approximately 8-15% of that in acetone powder prepared from human plasma preincubated without DDVP. The decreased cholinesterase activites in human plasma with 1 X 10^{-7} M to 1 X 10^{-5} M DDVP and in acetone powder prepared from human plasma preincubated with 1 X 10^{-3} M DDVP were recovered by the addition of 1 X 10^{-3} M pyridine aldoxime methiodide (PAM).

Erythrocytes AChE inhibition

Human erythrocyte AChE inhibited by organophosphorus exposure *in vitro* also. 38.8% to 76.7% inhibition of erythrocyte AChE activity occurred due to exposure of 1.33 to 1.67 mm cyclophosphamide.[28] In other study, human AChE activity has been estimated as 2.29- 2.55 KU/l for control erythrocyte and 2.47 inhibition after *in vitro* exposure of herbicide.[22]

AChE inhibitory kinetics

Kinetic parameters of human AChE gives a mechanism of AChE inhibition due to organophosphorus compounds. In other report Al-Jaferi *et al* [28] also reported K_m and V_{max} as 132µm and 73.8 µm/h/mg in control human erythrocyte by using the method described by Ellman *et al* [29] for radiometric assay. After the toxicity of cyclophosphamide (CP) the K m increased by 78% whereas V max decreased by 54.55.[30] Datta *et al* 1994 found phosphamidon and malathion to inhibit the activity of human AChE *in vitro*, in the human erythrocyte membrane. Lineweaver- Burk analysis indicated that the insecticide induced inhibition of AChE activity was uncompetitive in nature. Three sublethal doses of the organophosphate pesticide caused a significant inhibition of AChE.[31] The maximum inhibition was noticed at the highest dose. The enhanced inhibitory constant K_m and ACh contents in the heart muscles with the increase of dose showed inhibition of enzyme. The constant V_{max} showed competitive nature of inhibition. A significant inhibition of AChE (69%) indicated that DDVP is a strong inhibitor of enzyme in heart.

(V) Effect of antidotes on AChE inhibition

Several antidotes protects AChE inhibition caused by anticholinesterases. New bispyramidinium oximes HI6 and HLo7[10] have been used as promising antidotes against poisoning by highly toxic organophosphorus compounds i.e. nerve agents. The human AChE was inhibited (30 min) by chlorfenvinphos, dichlorvos, dicrotophos, heptonophos, mevinphos, monocrotophos, paraoxon, phosphamidon, trichlorfon, malaoxon, omethoate, oxydemeton-methyl or methamidophos by 85-98% of control. after removal of excess inhibitor, obidoxime, pralidoxime were added and AChE activity was measured (5-60 min). The oximes significantly but not completely inhibited AChE. The velocity and extent of reactivation were dependent on the oxime and its concentration. In all cases

obidoxime was superior to the three other oximes, followed by HLo7, 2-PAM and HI6 in reactivating human AChE activity inhibited by organophosphate pesticides.

Conclusion

Level of AChE inhibition, severity of behavioral symptoms in the human increases with increasing pesticide doses, exposure time, atmospheric temperature, developmental age, sex etc. Although, toxic effect of organophosphorus pesticides and carbamates etc are frequently observed *in vivo* and *in vitro* conditions with the clinical symptoms such as headache, lightheadedness, wheezing, shortness of breath, nausea, and fatigue. The alterations in K m and V max in tissues exposed to pesticides might be related to the changes in AChE kinetic mechanism. The intoxication of human is harmful in skin exposure, inhalation through respiratory tract and ingestion. To avoid the pesticidal toxicity biosensor should be used for the direct detection of pesticides in spiked tap water and fruit juices without any pretreatment steps.[4] In these cases, the LOD3 sigma of 1.5, 2.8 and 4 ng/ml paraoxon in tap water, orange juice and apple juice were obtained, respectively and they detected 0.2 ng/ml of paraoxon in less than 15 min. Limits of detection for other organophosphate (chlorpyrifos, diazinon) and carbamate (carbaryl, carbofuran) pesticides varied, depending on their antiacetylcholinesterase (AntiAChE) toxicity, from 1 ng/ml to 4 microg/ml.

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