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Pitfalls to Avoid while Interpreting Cholinesterase Activity Levels in Cholinesterase Inhibitor Pesticides Poisoning

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ABSTRACT

The cholinesterase activity (AcCh) assay finds an important place in the diagnosis of acute poisoning by cholinesterase inhibitor pesticides, allowing the indication and the efficacy evaluation of antidote treatment with atropine and oximes. AcCh is also a biomarker of effect in occupational exposure to cholinesterase inhibitor pesticides. However, some factors may disrupt AcCh levels and distort the interpretation of the assay results. Hence, the present review aimed to summarize the factors and the variations that may have an impact on the interpretation of AcCh. Indeed, butyrylcholinesterase and acetylcholinesterase are subject to wide physiological individual variations, such as to age, weight and height. Genetic and pathological state may also be factors influencing AcCh levels. The consumption of drugs and daily exposure to some toxicants may also disrupt the AcCh levels, either by direct action on the enzyme or by disrupting its synthesis. In addition, analytical variations and interferences are to be considered while interpreting the results. These variations could induce an underestimation or an overestimation of the cholinesterase activity levels and could lead to diagnostic errors. To conclude, the dosage of cholinesterase activity constitutes an important biomarker of effect in clinical and occupational toxicology. Its interpretation has to be done delicately, taking into consideration all the factors and variations that may influence it.

Key words: acetylcholinesterase, butyrylcholinesterase, cholinesterase activity, cholinesterase inhibitors, pathological variations, physiological variations

INTRODUCTION

Cholinesterase inhibitor pesticides are agricultural, public health and household insecticides that are widely used to control harmful insects. Despite their benefits, these products could expose the general population and workers handling them to potential health hazards from poisoning.

These insecticides include organophosphates and carbamates, some of which are potent inhibitors

of cholinesterases. They act by phosphorylating/ carbamylating the esterase site of the enzyme, thus opposing the physiological hydrolysis of their substrates, notably acetylcholine, and leading to its accumulation.¹

Two types of cholinesterases are differentiated according to their origin, structure, action specificity, and the indication of the measurement of their activity. Acetylcholinesterase (AChE), also called erythrocyte (globular) cholinesterase or true cholinesterase; has a specific affinity to its natural substrate, acetylcholine. It is synthesized in the blood cells and is found at synapses in nervous tissue and at the neuromuscular junction. Its physiological role is to ensure the functioning of cholinergic synapses, avoiding the accumulation of the neurotransmitter. It has a very fast activity and is able to hydrolyze 4000 molecules of acetylcholine per site and per second. The butyrylcholinesterase (BChE), also called plasma cholinesterase or pseudocholinesterase;

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could hydrolyze a large number of synthetic and natural esters (butyrylthiocholine, acetylcholine, and succinylcholine). BChE is present in the serum and in the liver where it is synthesized, but also in the pancreas, the intestine, and other tissues. Its physiological role is not fully known to date.²⁻⁵

Inhibition of these enzymes by cholinesterase inhibitor pesticides leads to the accumulation of acetylcholine at synapses in the central and peripheral nervous system, and at nerve endings leading to an overstimulation of cholinergic receptors, inducing cholinergic crisis, which is manifested by three syndromes: muscarinic, nicotinic, and central nervous system syndromes inducing neurological, electrolyte, cardiovascular and respiratory disorders that can lead to death.⁶ The decrease in AcCh levels in the blood constitutes a biomarker of effect, which finds an important place in the diagnosis of cholinesterase inhibitor pesticide poisoning, initially indicated by clinical assessment. The assay also helps to validate the use of a cholinesterase regenerating treatment.³

In addition to cholinesterase inhibitor insecticides, other factors may increase or decrease the AcCh levels, which could lead to confusion when interpreting the AcCh assay's results, especially when the clinical symptoms are not suggestive of cholinesterase inhibitor poisoning. Hence, the present review aims to emphasize the factors that may vary the AcCh levels (pathophysiological, drugs, toxicologic, and analytical) and their impact on the interpretation of these levels.

CHOLINESTERASE ACTIVITY VARIABILITY FACTORS

Physiological variability factors

In a study that lasted one year, the AChE activity varied considerably less than the BChE and appears to be relatively constant. In the absence of any pathological variation, the intra-individual variability of AChE activity can rise to 10%. However, inter-individual variability is more marked.^{7,8} In humans, there are no genetic variants that alter the catalytic activity of AChE.⁹

The intra-individual variability of BChE is much

greater and is related to age, gender, weight, and height. Indeed, in newborns it decreases by 50%, then it gradually increases until the age of 6 years. Between the ages of 3 and 6 years, it is 30% higher than in adults, then it decreases over the age of 70 years. This could be related to liver mass in young children. The lowest BChE activity is observed between the ages of 30-40 years and returns to pubertal levels by the age of 60 years. These differences are probably hormonal and influenced by variations in estrogen levels modifying hepatic protein synthesis.^{10,11} BChE activity increases with the weight and height of the individual; it has been reported that obese individuals have a high level of BChE.⁷

BChE level is also subject to wide inter-individual variations.^{7,12} No difference in plasma activity between gender was observed in individuals under 10 years of age.¹⁰ However, it is 10-15% higher in males than in females; it decreases in pregnant women and those who use contraception.^{7,10}

Indeed, during pregnancy, the decrease in BChE level begins approximately in the 10th week of pregnancy until postpartum. This decrease is 24% during pregnancy, 25% on the first day postpartum, and 33% on the third day postpartum. Then, it normalizes between 10 days and 6 weeks postpartum.¹³

Genetically, more than 40 mutations affecting the BChE gene have been identified¹⁴, which represents more than 237 genetic variants (homozygous or heterozygous).⁷ It is estimated that nearly 24% of the population carries at least one nucleotide variation within BChE.¹⁵ The most frequently studied and clinically relevant phenotypes are summarized in Table 1.¹³

Pathological variability factors

Many pathological factors could disrupt cholinesterase levels either by decreasing its level or by increasing it. Indeed, AChE may be increased in thalassemia and reticulocytosis produced by secondary polycythemia which increases blood cell production.⁸ Causes of decreased AChE activity are rare. AChE levels may be decreased in leukemia and some other cancers, as well as in Biermer's anemia

Table 1 Most common BChE genetic variants¹³

Genetic variants	Frequency	BChE activity
Wild (U)	More than 98% of the general population	Normal activity levels
Atypical or dibucaine-resistant (A)	Homozygous: 0.03-0.01% Heterozygous: More than 4%	Homozygous: cholinesterase activity decreased by 70%
Fluoride resistant (F)	Homozygous: 0.0007% Heterozygous: more common but clinically less significant	Cholinesterase activity decreased by 60%
Silent (S)	Homozygous: 0.01-0.008% Heterozygous: more common but clinically less significant	No cholinesterase activity
Kalow (K)	Homozygous: 1.5% (often combined with other variants)	Cholinesterase activity decreased by 30%

due to a decrease in the number of red blood cells, and depend on the number of blood cells.⁷

Several pathologies are responsible for the decrease in BChE:

Hepatic disease: BChE is mainly synthesized in the liver; when liver function is impaired, BChE synthesis is also disrupted. Decreased BChE levels have been observed in hepatitis, liver abscess, cirrhosis, and end-stage liver disease. Additionally, BChE activity may be decreased by 30-50% in acute hepatitis and by 50% in cirrhosis and chronic cancers.^{2,10,13,16-19}

Kidney disease: BChE decreases by 30-35% in patients with acute or chronic kidney injuries and in anephric patients. As the enzyme is dialysable, there is a significant decrease of BChE in the case of iterative plasma exchanges; in one session, there could be a 64% drop. It would appear that BChE is either destroyed or permanently and inexplicably inactivated by the institution of cardiopulmonary bypass (CPB). This inactivation persists up to de novo synthesis.^{10,13,16,17}

Malnutrition: decreased BChE levels are probably related to changes in hepatic protein (serum albumin) and enzyme synthesis, accompanying malnutrition.^{13,16,17}

Cancers: decreased levels of BChE have been associated with malignancies and carcinomas, with decreases closely related to primary lesion site and the

degree of spread. Hepatic carcinomas demonstrate the greatest degree of decrease, followed by lung, gastrointestinal, and genitourinary malignancies; breast cancer affects less BChE levels.^{10,13,20} The most probable hypothesis is the production in some types of cancers of BChE inhibitors.¹⁰

Burns: the size and severity of a burn are closely correlated with the decrease in BChE levels. The lowest levels were found 5-6 days after a burn, with reduction sometimes 80% or greater, and even up to 4 months afterwards in severely burned patients. It is suggested that the initial decrease in enzyme activity is due to the dilution effect and transcapillary losses, while the prolonged decline is due to decreased hepatic synthesis of the enzyme or its release and/or the presence of an inhibiting substance released by the burned tissues.^{10,13,16}

Other diseases: other diseases could also decrease BChE levels such as the occurrence of a syndrome associating hemolysis, elevated liver enzymes, and low platelet count (HELLP), post-operative severe sepsis or endotoxin infection (tetanus), leprosy, tuberculosis, typhus, collagen disease, rheumatoid arthritis, inflammatory disease, myocardial infarction, myxedema, debilitation, and anemia.^{2,7,10,13,16-18,20,21}

Although the mechanism of decreased cholinesterase levels in sepsis has not yet been determined, it is thought to be affected by infections and inflammatory processes.²² Additionally, it has

been hypothesized that cholinesterase synthesis decreases due to liver dysfunction related to disease progression, increased capillary permeability, increased cholinesterase catabolism, and inhibition of cholinesterase by inflammatory mediators such as cytokines.²³ This same mechanism has been proposed in the decrease in cholinesterase activity in COVID-19 pneumonia.²⁴

Some neurological diseases affect also the BChE level, namely epilepsy and neurodegenerative pathologies such as Alzheimer's disease and Parkinson's disease.^{10,25,26}

BChE levels could also increase in diabetics and patients with metabolic syndrome. It is increased in abnormal lipid metabolism by non-specific stimulation of protein synthesis in the liver and is correlated with the concentrations of LDL (low-density lipoprotein), VLDL (very low-density lipoprotein), cholesterol, and triglycerides. Nephrotic syndrome is the only circumstance where elevated BChE activity and hypoalbuminemia coexist.^{10,16,24,27}

BChE also increases in certain neuropsychiatric pathologies such as depression, autism, anxiety, and schizophrenia^{25,26}, as well as in asthmatics and hypertensives and in cases of alcoholism, psoriasis, nodular goiter, and thyrotoxicosis.¹⁰

Variability factors related to medication and toxicants

AChE is not greatly affected by drugs and toxicants. Carbamates, such as pyridostigmine, neostigmine, physostigmine, and rivastigmine that are used in the treatment of myasthenia gravis and Alzheimer's disease, could affect AChE levels by forming a carbamoyl complex with the serine residue of the AChE catalytic triad.²⁸⁻³⁰ Other carbamates have long been used as pesticides such as aldicarb, carbaryl, carbofuran, and propoxur could also act by inhibiting cholinesterases.³ It has also been described that AChE could be lowered after taking antimalarial treatments.⁷

The BChE can bind to a wide variety of compounds containing amines and quaternary ammoniums. These ligands act in a competitive, non-competitive or mixed way that can subsequently disrupt its

activity. Among the xenobiotics that can decrease the BChE level are organophosphate pesticides (OP) and carbamate pesticides, estrogenic contraceptives, ranitidine, bambuterol (antihistamine), monoamine oxidase inhibitor antidepressants (MAOIs), sertraline, lithium, benzamides, tiapride, metoclopramide, chlorpromazine, local anesthetics (procaine), halogenated anesthetics (enflurane, sevoflurane) or infusions (propanidide), muscle relaxant adjuvants (pancuronium), echothiophate (treatment of glaucoma), nitrogen mustards such as cyclophosphamide (cancer drug), radiation therapy, Alzheimer's treatments (tacrine, donepezil, galantamine, cymserine, huperzine, rivastigmine), myasthenia gravis treatments (neostigmine, pyridostigmine, physostigmine, edrophonium), natural alkaloids, and solanaceae extracts (tobacco).^{10,29,30}

Many molecules are able to inhibit the BChE activity in a reversible or irreversible way.

Reversible or short-acting agents interact with the enzyme near its catalytic site, without producing a covalent complex. They bind to the enzyme using weak bonds, similar to those used to attach the natural substrate. These bonds form quickly but break just as easily. Consequently, reversible inhibitors have an instantaneous action but do not have a permanent action. The duration of action of these agents is relatively short.^{29,31}

Some reversible BChE inhibitors are of therapeutic interest. For example, various benzamide-like dopamine receptor antagonists, such as metoclopramide, ranitidine and tiapride, have been found to protect cholinesterases against irreversible inhibition by potent OP compounds. These molecules could be used for the prophylaxis of OP poisoning.^{32,33} Molecules used in the palliative treatment of Alzheimer's disease such as tacrine, huperzine, donepezil, and galantamine, and the treatment of myasthenia gravis such as edrophonium are also reversible inhibitors of BChE.^{28,30}

However, pseudo-irreversible or intermediate-acting inhibitors covalently attach to the enzyme, and the bond is slowly broken. In this group, we find

Table 2 Examples of analytical conditions for cholinesterases assay^{35,36}

	BChE	AChE
Sampling	Venous blood on lithium heparin with gel	Venous blood on EDTA without gel
Precautions	Avoid contamination of the sample by OP (take samples away from contaminated areas and after careful cleaning of the skin)	
Transport	Can be done at room temperature	
Conservation	BChE is stable after centrifugation and separation for: <ul style="list-style-type: none"> • 7 days if stored at 2-8°C • Several weeks if stored at 0-5°C • Several months if plasma is frozen 	Analysis must be rapid either immediately or after brief refrigeration at 4°C

carbamates such as physostigmine and rivastigmine which has the same inhibitory concentration for BChE and AChE.^{28,29} Cymserine and its derivatives are analogs of physostigmine and act also as pseudo-irreversible inhibitors with selectivity for BChE.³⁴

Regarding irreversible inhibitors, their effect is based on phosphorylation (or phosphonylation) of the serine hydroxy group at the esterase site of the enzyme. The phosphorylated enzyme undergoes two reactions which are: spontaneous reactivation and dealkylation also called aging. The aged enzyme can no longer undergo spontaneous reactivation. Irreversible inhibitors react with the enzyme by forming a strong bond, usually covalent.²⁹

This class of inhibitors includes several organophosphorus compounds which can be pesticides (coumaphos, paraoxon, dichlorvos, demeton, malathion) or drugs (echothiophate, cyclophosphamide). Some organophosphates are powerful chemical warfare agents (tabun, sarin, soman, VX). Phosphorylated cholinesterases can be reactivated by nucleophilic compounds, oximes, which may be used in the emergency treatment of poisoning by these nerve agents.^{10,30}

Variability factors related to the sampling, conservation and analysis methods

The sampling, storage and analysis methods could induce variability of the AChE and BChE levels. To avoid this kind of variability which can induce errors of interpretation, the sampling, conservation, and analysis conditions must be respected (Table 2).

There is no standardized and universal approved laboratory technique or method for this type of

assay. This makes it prone to many pre-analytical and analytical errors. However, since 1961, international organizations have recommended the Ellman method. This is a rapid, high throughput assay method.^{37,38} However, several factors can influence the results. Indeed, the samples could be contaminated by the pesticides in the skin. Therefore, it must be carried out away from contaminated areas and after careful cleaning of the skin. Transport of the samples to the laboratory must be carried out under special conditions, namely a temperature of approximately 4°C for a maximum of 2 hours to prevent the enzymes inactivation. Regarding storage, the AChE activity remains stable in the laboratory for 7 days at -20°C, then it begins to slowly decrease, it reaches 91.8% after 34 days, while the BChE activity in plasma remains unchanged for 34 days.³⁷ The multiplicity of assay techniques, reagents and manipulators would be responsible for 20-40% of the variability of the results, even when used by an experienced laboratory.^{35,36}

Some solvents used in the preparation of reagents have been shown to interfere with cholinesterase activity. Dimethylsulfoxide and acetone, two commonly used solvents, were found to have serious inhibitory potential at a concentration of 5% by volume. Ethanol, methanol and acetonitrile have the lowest interfering potentials and are therefore considered the safest solvents.³⁸

CONCLUSION

The cholinesterase activity is the biomarker of choice to confirm the diagnosis of acute and

chronic poisoning with cholinesterase inhibitor pesticides. However, several factors could affect its levels. It is the responsibility of the biologist and the clinician to ensure a correct interpretation of its value by considering all the factors related to the analysis methods as well as the pathophysiological, medicinal, and toxicologic factors that could influence it.

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