Detection of Cholinesterase Inhibition

The Significance of Cholinesterase Measurements

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ABSTRACT

Human cholinesterase exists in two forms—acetylcholinesterase located in tissue microsomes and red blood cells and serum cholinesterase found in serum or plasma. The two enzymes display marked differences in structure, substrate specificity, biological function, and origin. Contemporary methods employ acylthiocholine as substrate for serum cholinesterase and a second coupled reaction of thiocholine and chromogenic disulfide agents. Clinical applications are primarily centered on subnormal levels of enzyme activity. The decreased activity levels can be caused by inhibitors, reduced biosynthesis, or dysfunctional genetic variants. Changes in enzyme activity should be related to baseline levels because there is wide individual variation as well as methodological variation. Once baseline levels have been established, cholinesterase activity becomes a sensitive indicator of pesticide intoxication and hepatic biosynthetic capacity. A more sophisticated assay, performed in the presence of an inhibitor, is required to detect the atypical genetic variants of serum or plasma cholinesterase.

Introduction

Cholinesterase is, by definition, an enzyme which can catalyze the hydrolysis of an ester of choline. Physiologically, the most important choline ester in the human body is acetylcholine, which is hydrolyzed by acetylcholinesterase. Acetylcholine is a chemical stimulant. By hydrolyzing acetylcholine released from nerve endings, acetylcholinesterase regulates nervous transmission between preganglionic fibers and autonomic ganglia, postganglionic cholinergic nerves and muscles, and transmission of nervous control to the adrenal medulla. The red blood cell contains the same enzyme, and the level of red cell activity is believed to reflect tissue activity.

Another cholinesterase of much broader substrate specificity, serum cholinesterase, is found in plasma and has no known biological function. However, its measurement has been useful to detect pesticide intoxication, loss of biosynthetic capacity of the liver, and unusual sensitivity to succinylcholine administration at the time of surgery.
Acetylcholinesterase

Acetylcholinesterase (acetylcholine hydrolase EC 3.1.1.7: AChE) is found in erythrocytes, lung, spleen, nerve endings, and the gray matter of the brain. Acetylcholine is released from the endings of cholinergic fibers in response to the conduction of an action potential along the fiber. The acetylcholine is produced under the influence of choline acetylase within vesicles that occur throughout the cell but are more numerous at the axonal endings. The release of acetylcholine at the junction between pre- and postganglionic fibers (synapse) or between a nerve fiber and an effector cell results in transmission of the impulse. Under normal circumstances, the acetylcholine released is hydrolyzed by AChE almost instantly and there is no accumulation of the ester. The rapid destruction accounts for the brevity and unity of each normal impulse. Thus, the normal function of acetylcholine depends upon its rapid destruction by AChE (figure 1).

If anything interferes with this destruction, acetylcholine tends to accumulate at the junctions where it is produced. A small excess produces great stimulation, but a further excess produces flaccid paralysis.4

Among the many compounds which inhibit serum cholinesterase, those most commonly seen are succinylolethine, organic phosphorus compounds, and carbamic acid derivatives. Succinylolethine chloride blocks nerve impulses and is used in medicine as a muscle relaxant. Organic phosphorus compounds and carbamates are esters used as insecticides and react with esterases to form enzyme-substrate complexes. In the case of carbamates, this is a reversible complex, but with the phosphorus compounds and succinylolethine, a stable complex is formed. All of these interactions with AChE result in accumulation of acetylcholine at the neuromuscular junction and produce muscular paralysis.

In the case of succinylolethine and carbamates, recovery is dependent upon circulating serum cholinesterase. This enzyme will rapidly destroy the inhibitor. However, with the organic phosphorus pesticides, the inhibition is irreversible and delayed until there is synthesis of new protein.10

Serum Cholinesterase

In addition to the AChE found in red blood cells, another type of cholinesterase is found in plasma or serum (acylcholine acylhydrolase, EC 3.1.1.8:ChE).6 This ChE, also called butyrylcholinesterase and nonspecific cholinesterase, is distinguished from acetylcholinesterase or “true” cholinesterase by its preference for butyrylcholine as a substrate and its greater sensitivity to inhibition by organophosphates6 (figure 2).

Butyrylcholinesterase has been found in almost all major systems of the body including the pancreas, liver, heart, vascular system, urogenital system, and the white matter of the brain. In spite of the widespread presence of the enzyme in humans and animals, no biological function has been clearly established.

Perhaps the distinguishing hydrolytic property between these two cholinester-
DETECTION OF CHolinESTerase INHIBITION

TABLE I

Types of Cholinesterases

<table>
<thead>
<tr>
<th>Name</th>
<th>Plasma</th>
<th>Red Blood Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butyrylcholinesterase</td>
<td>Pseudocholinesterase</td>
<td>True cholinesterase</td>
</tr>
<tr>
<td></td>
<td>Acetylcholinesterase</td>
<td>Acetylcholinesterase</td>
</tr>
<tr>
<td>Location</td>
<td>Cytosols</td>
<td>Tissue microsomes</td>
</tr>
<tr>
<td></td>
<td>Plasma</td>
<td>nerve and muscle</td>
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<tr>
<td></td>
<td></td>
<td>erythrocytes</td>
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<td>Heterogeneity</td>
<td>Many esterases</td>
<td>One enzyme</td>
</tr>
<tr>
<td></td>
<td>Many isoenzymes</td>
<td>One variant reported</td>
</tr>
<tr>
<td>Natural substrate</td>
<td>Fatty acyl esters</td>
<td>Acetylcholine</td>
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<tr>
<td></td>
<td>Aromatic esters</td>
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<tr>
<td>Test substrates</td>
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<td>Acetylthiocholine</td>
</tr>
<tr>
<td></td>
<td>Butyrylthiocholine</td>
<td>Methacholine</td>
</tr>
<tr>
<td></td>
<td>Benzoylcholine</td>
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</tr>
<tr>
<td></td>
<td>Succinylcholine</td>
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</table>

Comparison of Two Types of Cholinesterase

In table I are compared the two enzymes, their specificity, and their locations within the body. Butyrylcholinesterase is found in the cytosols and the plasma compartment, and the erythrocytic AChE is found in the tissue microsomes of nervous and muscle tissue.

There is great heterogeneity in the plasma enzyme, with 12 or more isomeric forms. The natural substrates for the plasma enzyme include fatty acyl esters and aromatic esters; thus, it has a much broader substrate specificity.

The plasma enzyme is synthesized in the liver, and patients with liver disease will usually have decreased levels. Assay of ChE can serve as a sensitive marker for the synthetic capacity of the liver if the patient's normal baseline level is known.

Methods for Estimating Serum Cholinesterase Activity

There has been a diversity of methods for the estimation of this enzyme, including manometric, electrometric, titrimetric, colorimetric, and gasometric. Early manometric methods followed the formation of the H⁺ ion by titration with a bicarbonate buffer and estimation of the release of CO₂. Potentiometric methods, such as the method of Michel, measured the decrease in pH during the course of a fixed reaction time. Other methods followed the change in pH by employing a pH indicator which changed color in the range of 6.0 to 8.5. This color change, measured spectrophotometrically, was a measure of enzyme activity.

Contemporary methods employ acylthiocholine esters as substrates. These substrates are hydrolyzed at approximately the same rate as choline...
Figure 2. Cholinesterase reaction: Butyrylcholinesterase catalyzes the same type of reaction with butylthiocholine as the substrate. The plasma enzyme will accept benzoylcholine as a substrate but cannot hydrolyze acetyl-B-methylcholine. The red cell enzyme (AChE) displays a very narrow substrate specificity, accepting only acetylthiocholine and methacholine.

Esters and the thiocholine formed can be measured by reaction with chromogenic disulfide agents such as DTNB, Ellman’s reagent [5,5’-dithiobis(2-nitro-benzoic acid)]. The reaction of butyrylthiocholine and DTNB (as the chromogen) is shown in figure 3. The color can be measured at 410 nm. The rate of color production is followed as a function of time and has good sensitivity and acceptable precision (less than 10 percent). The sample requirement is small, 10 μl, and results are expressed in international units. It is suitable for the assay of large numbers of clinical samples.

A similar ChE reaction employs another chromogen, 2,6-dichlorophenolindophenol. This blue dye is reduced to a colorless form upon reaction with the free sulphydryl group. This is an acceptable method for hospital laboratories.

In general, assays requiring long incubations are not recommended in cases of poisoning, owing to the possibility of spontaneous reversal.

Atypical Genetic Variants

The existence of the “atypical” variant of human serum ChE was first recognized in the early 1960s when succinylcholine (suxamethonium) was given during anesthesia and produced severe apnea. Succinylcholine competes with acetylcholine for receptor binding at the neuromuscular junction. It is not hydrolyzed by acetylcholinesterase, and its action persists until it is destroyed by plasma pseudocholinesterase.

In normal individuals, this drug induces muscle relaxation, and its action is short lived with spontaneous respirations returning within 2 to 10 minutes. However, in one out of every 2500 patients, clinically significant prolonged apnea occurs, persisting for minutes or even extending to several hours.

The majority of patients with sensitivity to succinylcholine have inherited abnormal pseudocholinesterase variants. Their ChE is usually resistant to inhibitors such as dibucaine. The atypical enzyme is estimated by using benzoylcholine substrate in the presence of the dibucaine inhibitor.

There are at least four different ChE variants: (1) Normal EuEu, (2) Dibucaine resistant EaEa, (3) Fluoride resistant EfEf, and (4) Silent gene EsEs (absent activity). The variants most commonly encountered are EaEa and EuEu. When a person with prolonged succinylcholine apnea has one of the variant enzymes, the total serum ChE activity is usually low. However, prolonged apnea may occur in those with EuEu and EaEa enzyme variants despite normal serum ChE activity.

Patients who are heterozygous usually produce enough enzyme to protect themselves against succinylcholine sensitivity. Those who are homozygous for the abnormal genes show various degrees of sensitivity depending upon their phenotype. Measurement of total serum cholinesterase as well as determination of the dibucaine number and fluoride number are needed to characterize fully cholinesterase variants. The latter
parameters indicate the percentage of inhibition of enzyme activity toward specified substrates.

**Reference Intervals**

Unfortunately, cholinesterase activity is expressed in different units when measured by different methods. These units are not strictly convertible because the kinetics are not identical. The results of any method can be expressed as a percentage of normal and compared with values determined by any other method. However, these conversions must be interpreted with caution. Ide-
ally, the same laboratory should be used for all enzyme measurements on the same individual.

There is normal physiological variation in ChE activity, and the coefficient of variation may vary from five to 12 percent and more. In spite of the statistical regularity in the ChE values of any unexposed, normal individual, that person may show striking and unpredictable variation from one sample to another. Maximal fluctuations of 20 to 23 percent in plasma values have been reported. Therefore, it is imperative to establish baseline values for each individual by replicate samplings prior to exposure.

Cholinesterase activity of plasma is higher in men than women. In the age range of 18 to 35 years, males have values about 1.5 times greater than women. Male levels decrease with aging and reach the female mean by age 70.

Clinical Significance

The finding of a low serum ChE level could indicate possible insecticide poisoning, compromised liver function, or atypical genetic variants. However, the test is not specific for these conditions, and the serum cholinesterase level may also be low in the following clinical settings: acute hepatitis, cirrhosis, advanced carcinoma, pregnancy (first trimester), myocardial infarction, pulmonary embolism, acute inflammatory states, and post-surgical nephrosis.

Pesticide Poisoning

Organophosphate compounds are highly toxic, and poisonings occur often in agricultural communities, greenhouses, and laboratories where they are used. The majority of the cases involving field workers come from two states, California and Florida. Workers engaged in agriculture and those working in organic chemical industries are subject to poisoning by inhalation of these materials or by direct skin contact. Among the organic phosphorus compounds that inhibit activity are insecticides, such as parathion, and tetraethyl pyrophosphate.

Acute organophosphate poisoning may cause blurred vision, dizziness, weakness, disorientation, headache, nausea, vomiting and muscle and abdominal cramps. Chronic subclinical exposure may result in sensory and motor peripheral neuropathy, manifested by generalized weakness and ataxia. If sufficient toxin is absorbed to inactivate all the acetylcholinesterase of nervous tissue, death will result.

Both cholinesterases are inhibited by insecticides. The activity of the serum enzyme falls more rapidly than does the red cell enzyme. In acute poisoning, the ChE and AChE activities are 30 to 50 percent of normal by the time symptoms appear. Chronic exposure may result in no clinical effects and can only be recognized by monitoring the enzyme levels and demonstrating enzyme inhibition.

The appearance of symptoms depends more upon the rate of fall of ChE activity than the absolute level of activity reached. Workers may show 70 to 80 percent inhibition of ChE after several weeks of moderate exposure without manifesting cholinergic symptoms. Most people must drop to greater than 80 percent inhibition before serious neuromuscular effects become apparent. On the other hand, a previously unexposed individual may develop symptoms after sudden exposure associated with a rapid drop in ChE activity of less than 30 percent from baseline.

The normal ranges are very wide. Patients may lose half of their ChE activity and still have values within the reference interval. For this reason, baseline values for ChE activity should be determined in all workers with a high risk of
exposure to organophosphates. A drop of 30 percent from baseline activity would indicate toxicity even though the level remains within the normal limits.

The California State Department of Health Services Program recommends that baseline values be determined after 30 days of no exposure. Two basal tests should be performed at least three days apart, but not more than 14 days. A third sampling is performed if the first two results show a difference of more than 20 percent. That person's baseline level is taken as the average of these two or three values.

There are no federal guidelines for biological monitoring of exposure to organic pesticides. California is the only state to mandate medical surveillance for agricultural workers. The program established in 1977 recommends medical surveillance of all applicators, loaders, mixers, and formulators if they have over 30 hours exposure in 30 days. Medical surveillance includes baseline red blood cell acetylcholinesterase and plasma cholinesterase determinations and subsequent periodic testing at the discretion of the physician.

For workers exposed to insecticides, the minimum retesting frequency is once at the peak of the season. However, weekly retesting is recommended for those people working with parathion. A drop of 30 percent from baseline necessitates re-testing and monitoring; a drop of 50 percent from baseline removes the patient from further exposure until ChE values return to pre-exposure range.

Recovery from organophosphate-induced cholinesterase inhibition is prolonged because phosphorylation results in formation of a stable chemical bond between the organophosphate compound and the enzyme. Plasma ChE will return to baseline activity within four to six weeks, and AChE activity will recover at a rate of approximately one percent per day.

Compromised Liver Function

There is evidence to indicate that the plasma ChE is produced by the liver in man and animals. The ChE activity appears to be related to the synthetic activity of the liver cells, similar to albumin. In the absence of known inhibitors, any decrease in serum activity reflects impaired hepatic biosynthesis.

Furthermore, changes in serum enzyme activity have been observed in hepatic diseases. A 30 to 50 percent decrease is observed in acute hepatitis, and even greater reductions are associated with chronic liver diseases such as cirrhosis and kwashiorkor.

Although the serum cholinesterase activity would provide a sensitive measure of the synthetic capacity of the liver, it requires comparison with a baseline level and this is seldom known. Thus, there has been limited application of the serum enzyme assay as a test of liver function.

Conclusions

Cholinesterase provides an excellent model for biochemical disease since there are genetic variants which produce an imperfect product. Although possession of this variant is usually benign, it may become manifest at the time of surgery.

The enzyme determination has also been used to study the reaction mechanism for esterase activity and the nature of the active site. On a more practical note, the enzyme levels may be measured to monitor exposure to pesticides and preclude disease.

References


