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Activity of cholinesterases in a young and healthy middle-European population: Relevance for toxicology, pharmacology and clinical praxis

Jana Zdarova Karasova[a,b,*], Zuzana Maderycova[b], Martina Tumova[b], Daniel Jun[a,b], Vit Rehacek[c,b], Kamil Kuca[a,d], Jan Misik[a,b]

[a] Biomedical Research Center, University Hospital, Hradec Kralove, Czech Republic
[b] Department of Toxicology and Military Pharmacy, Faculty of Military Health Sciences, Hradec Kralove, Czech Republic
[c] Transfusion Department, University Hospital, Hradec Kralove, Czech Republic
[d] Department of Cellular Biology and Pharmacology, College of Medicine, Florida, International University, Miami, USA

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ABSTRACT

The activity of human cholinesterases, erythrocyte acetylcholinesterase (AChE; EC 3.1.1.7) and plasma butyrylcholinesterase (BChE; EC 3.1.1.8) represents an important marker when monitoring exposure to pesticides/nerve agents, and may also be used in occupational medicine in diagnosis and prognosis of some diseases. In this study "normal/baseline" AChE and BChE activity has been investigated in a young and healthy population, with subsequent evaluation of several intra-population factors including sex, age (categories 18–25, 26–35 and 36–45 years old) and smoker status.

The modified Ellman's method was used for enzyme activity assessment in 387 young and healthy individuals (201 males and 186 females aged 18–45). A significant inter-sexual difference in AChE and BChE activity was found (AChE: 351 ± 67 for males and 377 ± 65 for females, (μmol/min)/(μmol of hemoglobin), $p < 0.001$; BChE: 140 ± 33 for males and 109 ± 29 for females, μkat/l, $p < 0.001$; mean ± SD). Despite the finding that mean AChE activity somewhat decreased whereas BChE activity grew within the age categories of the tested subjects, no significant effect of age on cholinesterase activity was found ($p > 0.05$). Smoking influenced cholinesterase activity – AChE activity in smokers was elevated (approx. 3% in males; 8% in females) relative to that in non-smokers ($p < 0.05$). Smoking was found not to have any effect on BChE activity.

Reference values based on confidence intervals for AChE and BChE activity were established. The presented results might be useful in routine clinical practice where the monitoring of blood AChE and plasma BChE activity is crucial for prognosis and diagnosis of organophosphate poisoning, in occupational medicine and in relevant mass casualty scenarios.

1. Introduction

Cholinesterases are polymorphic carboxylesterases displaying broad substrate specificity. There are two types named according to their localization in blood as red cell acetylcholinesterase (AChE; EC 3.1.1.7) and plasmatic butyrylcholinesterase (BChE; EC 3.1.1.8) (Taylor and Radić, 1994; Pohanka, 2011). AChE inhibition results in accumulation of the neurotransmitter acetylcholine in cholinergic synapses (autonomic nervous system, CNS and neuromuscular junctions) with typical signs of intoxication (Marrs, 1993; Maxwell et al., 2006; Peter et al., 2014). Acute BChE inhibition does not seem to cause critical features (Lockridge, 2015).

Both cholinesterases may be used as potentially stable biomarkers sensitive to selected inhibitors including organophosphorus nerve agents and pesticides (OPs) as well as some therapeutics (Munro et al., 1994; Kim et al., 2010; Ramírez-Santana et al., 2015; Cacciatore et al., 2015; Karasova et al., 2016). Among all the cholinesterase inhibitors, intoxication by organophosphorus pesticides is an important clinical issue around the World (Eddleston et al., 2002; Bertolote, 2006; McCauley, 2006; Gunnell et al., 2007). The symptoms of OP intoxication may be difficult to recognize in some cases when the etiology is unknown, so it is important to quantify a pre-exposure/baseline cholinesterase activity in the healthy population (Eddleston et al., 2008a; Peter et al., 2014). It is generally accepted that the degree of blood cholinesterase inhibition is related to the severity of the exposure and accompanying symptoms (bronchospasm, convulsions, vomiting, salivation, lacrimation, etc.). Thus, determination of the cholinesterase status in the case of intoxication is crucial for the early diagnosis of

* Corresponding author at: Department of Toxicology and Military Pharmacy, Faculty of Military Health Sciences, Trebesska 1575, 500 01 Hradec Kralove, Czech Republic.
E-mail address: zdarova.jana@gmail.com (J.Z. Karasova).

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exposure to inhibitors and for further monitoring of the therapeutic efficacy of oxime (AChE reactivator) treatment (Eddleston et al., 2008b). These results are important for rapid and precise decision-making.

The measurement of peripheral cholinesterase activity may also be valuable in occupational medicine, and in diagnosis or prognosis in illness. The relationship between peripheral cholinesterase activity and brain injury/illness is most often explained by the activation of the "cholinergic anti-inflammatory pathway". In the case of traumatic brain injury (TBI), correlation was found between serum cholinergic parameters and TBI severity, infective status, survival, and cognitive and neurofunctional outcomes, providing useful diagnostic and prognostic insights into TBI (Zhang et al., 2015). Also, certain relationships between peripheral AChE activity and brain Aβ plaques have been proved in Alzheimer disease patients. The link between brain Aβ and the peripheral cholinergic activity may also be explained by the "cholinergic anti-inflammatory pathway" (Alkalay et al., 2013).

Unfortunately, there is a general confusion in interpretation of AChE and BChE assays. Some OPs and other cholinesterase-inhibiting compounds used in industry inhibit BChE preferably to AChE. Thus, BChE may be used as a more sensitive marker of poisoning despite the fact that BChE inhibition does not relate to poisoning severity at all (Eddleston et al., 2008a; Lotti, 2001). It is also a useful marker for assessment of the elimination process in the warm-blooded organism. In contrast to BChE, the red cell AChE is currently considered as the major marker of poisoning severity and the need for atropine/oxime treatment (Katalinic et al., 2015).

The variability in cholinesterase measurement assays (electrometry, pH-stat, radiometry and colorimetry) is another problem in the clinical result interpretation. The colorimetric Ellman's method which is broadly preferred in occupational health screening represents a rapid, simple and cheap assay (Ellman et al., 1961). However interpretation of the results is rather unreliable due to variability in the method routine, especially in sample preparation, time of incubation and disturbances by the sample matrix. An appropriate understanding of Ellman's method limitations is essential for precise interpretation of the results.

The main aim of this study was the assessment of "normal/baseline" AChE and BChE activity in a young and healthy population, with subsequent evaluation of several intra-population factors including sex, age and smoking. Cholinesterase activity evaluation in whole blood and plasma was carried out robustly via a modified Ellman's method (Worek et al., 1999). This method was previously proved to have a high sensitivity and applicability in the therapeutic monitoring of organophosphate pesticide-poisoned patients. Moreover, the effect of two substantial factors – individual hemoglobin (Hb) levels and temperature during the *in vitro* assessment of cholinesterase activity was evaluated in this study. It was proved that both these factors have a significant impact on observed cholinesterase activity, and their importance for this methodology is considered.

Finally, the presented results might be useful in routine clinical practice where the monitoring of blood AChE and plasma BChE activity is crucial for prognosis and diagnosis in occupational medicine or in mass casualty scenarios.

2. Methods

2.1. Chemicals

Phosphate buffered saline tablets, acetylthiocholine iodide (ASCh), butyrylthiocholine iodide (BSCh), 5,5́-dithio-bis-2-nitrobenzoic acid (DTNB, so-called Ellman's reagent), Triton X-100, ethopropazine hydrochloride and huperzine A were purchased from Sigma Aldrich (Prague branch, Czech Republic).

2.2. Reagents

Phosphate buffer (PB; 0.1 M, pH 7.4) was obtained by dissolution of one tablet in 200 ml purified water and stored refrigerated at 4 °C for up 5 days for further use. Reagent for hemolysis of whole blood samples was prepared from PB and Triton X-100 (300 µl Triton X-100/1000 ml PB; Worek et al., 1999). Diluting reagent was stored at 4 °C for up to 5 days.

DTNB reagent (color reagent; 5 mM) was obtained by dissolution of 396.3 mg DTNB in 200 ml purified water by magnetic stirring at 40 °C with protection from sunlight, and stored in 8 ml aliquots at –20 °C.

Substrates (ASCh; 28.3 mM and BSCh; 63.2 mM) and a solution of ethopropazine (BChE inhibitor; 6 mM) were prepared as described elsewhere (Worek et al., 1999).

A solution of huperzine A (AChE inhibitor; 2 mM) was prepared by dissolution of 7.27 mg huperzine A in 10 ml purified water, and split into 0.5 ml aliquots and stored at −20 °C.

2.3. Subjects and sampling

Whole blood dilutions (blood hemolysis) were prepared immediately from venous heparinized blood samples taken from 387 healthy donors aged between 18 and 45 (29 ± 8 years old). Donors were chosen in cooperation with the Transfusion Department of University Hospital in Hradec Kralove. Before the analysis, each donor was assigned to an appropriate category of sex (male/female), age (18–25, 26–35, 36–45 years old) and smoking habit (non-smoker/smoker). All donors signed the Informed Patients Agreement and the study was approved by the ethical committee of University Hospital in Hradec Kralove.

Whole blood samples were hemolyzed by adding 100 µl heparinized blood to 3900 µl diluting reagent. Following careful mixing, the samples were analyzed immediately. Plasma samples were obtained from part of the heparinized blood after centrifugation (500*g*, 10 min, 10 °C; U320R, Boeco Germany), and were stored in 0.5 ml aliquots at −80 °C until the analysis.

2.4. Apparatus

The activities of the cholinesterases were measured using spectrophotometer (model Helios Alpha, Thermo Fisher Scientific, branch Czech Republic). Total Hb was determined in the laboratories of Transfusion Department of University Hospital in Hradec Kralove by standard Hb-assessment (model Cell-Dyn 3200; Abbott Laboratories, USA). The final dilution of whole blood during the procedure was 1:218 (v/v).

2.5. Enzyme activity determination

The standard procedures for AChE and BChE activity assessment are summarized in Table 1. The activity of both cholinesterases was measured at 436 nm to reduce interference by 412 nm Hb absorbance. The mixture was incubated for 10 min in the cuvette to achieve temperature equilibration, complete reaction of matrix sulfhydryl groups with DTNB, and complete inhibition of cholinesterase by ethopropazine or huperzine A. Following the incubation, specific substrate was added and the spectrophotometric procedure started. The measurement of color development was recorded for 3 min. The enzyme activity of all samples was assessed in duplicate (Worek et al., 1999). Assay validation such as assay linearity, within-run reproducibility, between-run reproducibility, activity calculation and AChE and BChE stability tests have been described previously (Worek et al., 1999).

In the second part of this clinical study the BChE activity was measured in two ways. The first was the commonly used method of plasma BChE assessment and the second was assessment using a selective AChE inhibitor (Huperzine A) in whole blood. The total AChE