

## **Collecting and measuring cholinesterase samples: avoiding the pitfalls.**

Horst Thiermann<sup>1</sup>, Franz Worek<sup>1</sup>, Peter Eyer<sup>2</sup> <sup>1</sup>*Bundeswehr Institute of Pharmacology and Toxicology, Munich, Germany;* <sup>2</sup>*Walther Straub Institute of Pharmacology and Toxicology, Ludwig Maximilians University, Munich, Germany*

The determination of cholinesterase activities is important in the monitoring of exposure to anticholinesterase agents, which are used as pesticides, nerve agents and drugs as in Alzheimer's disease. The agents comprise organophosphorus compounds (OP), carbamates and reversible inhibitors such as huperzine A and tacrine. Studies on the toxic mechanisms of these agents have historically focused on their interactions with serine hydrolases, particularly acetylcholinesterase (AChE, EC 3.1.1.7). AChE is found mostly in nervous tissue, where its major function is inactivation of the neurotransmitter acetylcholine in the synaptic cleft, and curiously in the membrane of red blood cells (RBCs), where its function is not fully understood. Generally, primates have the highest activity, rats one tenth and cats roughly 1% compared to human RBCs. The contribution of AChE in blood cells other than erythrocytes is negligible, because the relative amounts of these cells is several orders of magnitude less than that of RBCs. In contrast to other species, human plasma contains less than 2% of true AChE in whole blood. In human plasma, butyrylcholinesterase (BChE, EC 3.1.1.8) originating mainly from the liver is capable of splitting several cholinesters, including acetylcholine, the highest activity being found with butyrylcholine. There is considerable confusion in literature; authors often report that they had measured AChE in human plasma with acetylcholine as substrate where in fact they had measured the activity of BChE!

In blood samples of OP poisoned patients, reactions between AChE, OP and oximes will continue if the specimen is left at room temperature; inhibition, reactivation and ageing *ex vivo* may falsify the results. To avoid these reactions, immediate dilution of whole blood samples after withdrawal was considered a feasible method. We recommend the dilution at bed-side of 0.2 ml of whole blood (EDTA or heparin blood to inhibit clotting) into 20 ml of ice-cold distilled water in poly vials as used for scintillation counting. After proper mixing the vials should be brought immediately into a freezer (-20°C). By this manoeuvre secondary reactions are largely prevented and samples can be safely stored for some months prior to analysis. It should be emphasised that dilution with saline or buffer and intermediary cooling in the ice-box of a normal fridge (-5 °C) can result in settling of the RBCs before freezing, which may thwart the intention to inhibit further reactions *ex vivo* by dilution.

Convenient colorimetric determination of cholinesterase activities usually employs thiocholine esters. Liberation of thiocholine is detected with Ellman's reagent which yields a yellow product upon reaction with thiols. Several modifications of the original method have been recommended to avoid pitfalls: (i) The massive absorbance of oxyhaemoglobin interferes with the absorbance of the coloured indicator. Hence it is wise to change the detection wavelength from 412 to 436 nm to reduce the haemoglobin absorption. (ii) To further increase the signal-to-noise ratio, we reduced the spontaneous substrate hydrolysis by lowering the pH and reducing the substrate concentration. (iii) AChE and particularly BChE activities are quite temperature dependent thus requiring a constant temperature during determination. (iv) When AChE activity is measured in diluted whole blood, a selective BChE inhibitor such as a phenothiazine derivative, e.g.

ethopropazine, is indispensable.

(v) Reactions of matrix sulfhydryls with Ellman's reagent are interfering. While the reaction with glutathione is very fast and usually complete before starting the reaction with acetylthiocholine, the sluggish reaction with sulfhydryls of haemoglobin may be annoying. Preincubation with Ellman's reagent during temperature equilibration avoids this interference. (vi) Dilution errors and fluctuations in blood volume during resuscitation are compensated when AChE activity is referred to haemoglobin which is determined as cyanomethaemoglobin. In doing so, normal RBC-AChE activity is found to be quite constant with 90% of the values falling between the limits of 500 and 700 mU/ $\mu$ mol Hb(Fe). In contrast to RBC-AChE, BChE activity shows much larger variations, both intra- and inter-individually. Several phenotypes of BChE are expressed with silent mutants being found more frequently in some Indian communities. Hence, exposure monitoring using only BChE activity requires individual pre-exposure data.

Confirmation of poisoning, monitoring of patients and evaluation of the efficacy of reactivators is best performed by a test battery to assess the cholinesterase status. This array comprises the determination of AChE and BChE activities in whole blood dilution and plasma, respectively. The suitability of oxime treatment can be assessed by testing the reactivatability of the patient's AChE, determined after incubation of the diluted RBC-AChE with high oxime concentration, e.g. 0.1 mM pralidoxime for 30 min. Unknown inhibitory material in patient's plasma can be assessed by incubating plasma with test RBCs of known activity. These results provide a meaningful tool for defining the necessity and duration of oxime treatment in OP poisoned patients. It should be emphasized, however, that these results are only meaningful if blood was collected from a vein (preferably of the collateral arm) not distally used for oxime infusion! Meanwhile a battery-powered Ellman-based test-system is on the market which automatically compensates for various ambient temperatures and produces test results in less than 5 min from a 10  $\mu$ l whole blood finger-stick sample. This system can also be adapted for using diluted blood samples that are processed as outlined above. It is hoped that reliable determinations of RBC-AChE on the spot may help physicians to decide whether oximes are necessary and potentially useful.

**References:** Worek, F., Mast, U., Kiderlen, D., Diepold, C., Eyer, P. Improved determination of acetylcholinesterase activity in human whole blood. *Clin Chim Acta* 1999;288:73-90.

Worek, F., Koller, M., Thiermann, H., Szinicz, L. Diagnostic aspects of organophosphate poisoning. *Toxicology* 2005;214:182-9.