Analyzing cholinesterase in organophosphate poisoning

The German army automates the determination of AChE activity in whole blood

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Agrochemicals belonging to the organophosphate group are used extensively around the world, due to their efficacy and comparatively low environmental persistence. They are, however, highly toxic and present a considerable risk to humans, as demonstrated by the estimated 3 million acute pesticide poisonings and 200,000 fatalities globally every year. A few particularly poisonous organophosphates, including soman, sarin, tabun and VX (known as nerve agents), were developed as chemical weapons and have even been used recently, notably in the Iran-Iraq war and the Tokyo underground in 1995, despite being outlawed around the world.

Organophosphates inhibit serine esterases, particularly acetylcholinesterase (AChE), by covalently binding to the enzyme and disturbing its physiological function – i.e. preventing cleavage of the neurotransmitter, acetylcholine (ACh), by AChE. This leads to an accumulation of ACh in cholinergic synapses of both the central and peripheral nervous systems and at the neuromuscular junction, resulting in hyperexcitation and subsequent paralysis of the target organs and, eventually, death due to central and peripheral respiratory paralysis[1,2].

Organophosphate poisoning can be treated by immediate administration of atropine and AChE reactivators (oximes; Obidoxime in Germany, pralidoxime in the UK and USA) but it is necessary throughout treatment to measure and monitor levels of the affected enzyme within the body. Muscular AChE is not easily accessed in patients, so erythrocyte AChE and plasma cholinesterase (butyrylcholinesterase; BChE) are analyzed instead.

The Bundeswehr Institute of Pharmacology and Toxicology co-operates with the South Asian Clinical Toxicology Research Collaboration Centre for Tropical Medicine, University of Oxford, England[3] in a prospective cohort clinical trial in Sri Lanka. Blood samples from patients are analyzed in the Institute for AChE activity, BChE activity and hemoglobin. Until recently, the parameters have been analyzed manually by measuring a 3 ml sample using a standard commercial spectrophotometer with a temperature-controlled cuvette holder and water bath at 37°C. However, this manual procedure quickly reached its limits when analyzing large numbers of samples and, to solve this problem, an automated microplate method was developed to process multiple samples in parallel. Tecan's Genesis 150 workstation was introduced, with automated pipetting and a robotic arm as well as an integrated spectrophotometer, a 37°C incubator, temperature-controlled microplates racks and cooled sample preparation (figure 1a). Adapting the protocol for automation was relatively straightforward because the parameters used previously could almost all be translated and known incubation times, wavelengths and measuring times were not altered. This application can be automated on other Tecan liquid handling workstations, including from the Freedom EVO® series.

Cholinesterase activity is measured in a whole blood sample collected from the patient and immediately diluted and cooled to slow down any *ex vivo* reactions that can rapidly take place between AChE, organophosphates and oximes. Cholinesterases convert specific substrates and, in the conversion process, thiocholine is formed as a product (figure 1b). This product reacts with DTNB (5-5'dithio bis-(2-nitrobenzoic acid)) present in the sample to give a yellow dye that can be measured with a spectrophotometer; the color development over time serves as a direct measure of the enzyme's activity. The hemoglobin levels in the dilutions are also measured, using Zijlstra's method[4], to allow correction of any diluting errors in preparation of the samples; the AChE activity can be correlated with the hemoglobin levels[5,6].

To validate this set-up, extensive comparative measurements were made using both the automated and manual methods. AChE activity was analyzed in aliquots of native donor whole blood that

were inhibited with 100 nM soman; BChE activity in plasma harvested from soman-inhibited blood samples was measured and hemoglobin levels were determined in dilutions of whole blood samples with different proportions of erythrocytes and added plasma. In addition, the reactivation capability of whole blood inhibited with either 100 nM soman, which does not allow AChE reactivation, or 200 nM paraoxon-ethyl (PxE), which readily allows AChE reactivation, was analyzed and the residual inhibitor was also measured in donor plasma samples inhibited with five different concentrations of paraoxon-ethyl. The results obtained with both methods correlated very strongly in all measurements.

Once validated, the automated method was used to analyze and monitor AChE and BChE activity in samples from the Sri Lanka patients who had attempted suicide by oral ingestion of pesticides (quinalphos or fenthion), (figures 2 and 3). Whole blood samples were collected from the patients (t = 0) and oxime therapy was started with pralidoxime (bolus dose and infusion therapy); further samples were collected within a predefined time window. Quinalphos results in diethylphosphorylation of AChE, this enzyme species can be readily reactivated (figure 2) and so, four hours after admission to the hospital, no residual inhibitor remained in those affected patients. Fenthion results in a dimethylphosphorylated enzyme that ages quickly and cannot be reactivated (figure 3), meaning that in patients who had ingested this pesticide, no significant increase could be recorded in AChE activity, and BChE activity remained completely inhibited.

The determination of cholinesterase status is primarily intended for analyzing patients poisoned with organophosphates (accidental or suicidal poisoning), but it can also be applied after the use of nerve agents in military conflicts or terrorist attacks, as well as in the context of occupational medical examinations.

Figure 1a: Tecan GENESIS 150 workstation. The workstation is an open source system and can also be used now for kinetic samples. It has an integrated Sunrise™ spectrophotometer and uses TOPS™ 4.0 with Magellan

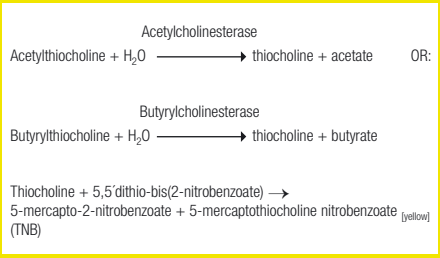

Figure 1b: Formation of DTNB produces a yellow dye that serves as a direct measure of cholinesterase activity

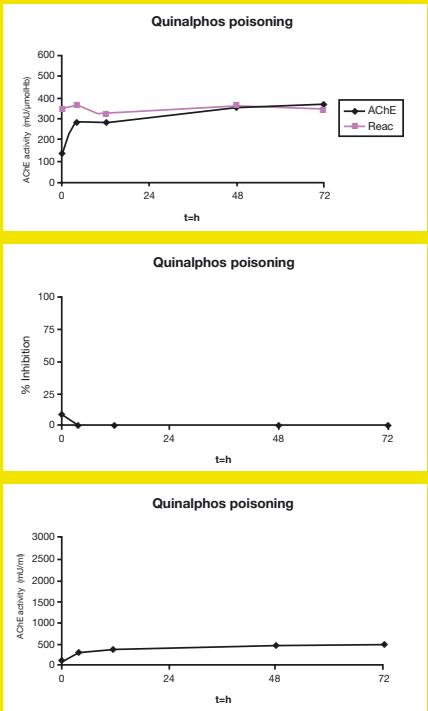

Figure 2: Example of Quinalphos poisoning. The reactivation capability shows the maximum achievable AChE activity. The per cent inhibition shows that no residual inhibitor remains after 4 hours

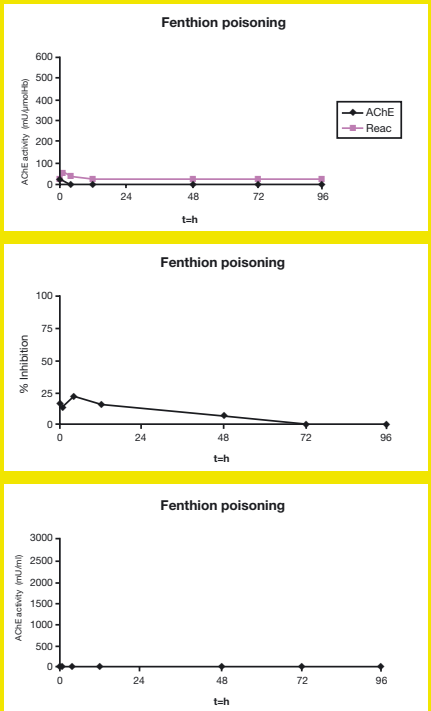

Figure 3: Example of Fenthion poisoning. Due to ageing, reactivation is almost impossible (top). In spite of a decline in residual inhibitor (middle) no significant increase in AChE activity is recorded (top). BChE activity is completely inhibited (bottom)

References

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