Editorial

Cholinesterase estimations revisited: the clinical relevance

In 1986, Whittaker wrote 'Churchill-Davidson's editorial entitled "The succinylcholine story" in 1963, highlighted probably for the first time in the UK, the role of serum protein polymorphism in drug sensitivity' [1]. Following a series of detailed family studies, in 1959 Kalow [2] concluded that individuals who had prolonged apnoea following succinylcholine administration were homozygous for an atypical form of cholinesterase, the product of a gene other than that found in unaffected people [3]. This led to the introduction of the term 'pharmacogenetics', and cholinesterase estimations became an important biochemical investigation in anaesthesia. Subsequently, the global burden of pesticide-related disease – particularly due to the potent inhibitors of cholinesterase activity, the organophosphates – increased the importance of quantifying the activity of this enzyme in clinical practice. Most of the ill-health associated with exposure to organophosphates has been attributed to inhibition of the enzyme acetylcholinesterase in a range of nerve, neuromuscular (skeletal, smooth, cardiac) and glandular tissues where the enzyme plays a key role in cell-to-cell communication.

The existence of an esterase capable of hydrolyzing acetylcholine was suggested by Dale [4] and established by Loewi and Navratil [5]. The term 'cholinesterase' was proposed by Stedman and colleagues [6] for this enzyme. Subsequently, Alles and Hawes [7] demonstrated that cholinesterase in human erythrocytes differed in a preferred substrate from that in human plasma. The erythrocyte enzyme – and the enzyme in conductive tissues (in all excitable tissues: cholinergic or adrenergic, motor or sensory, peripheral or central nerve fibres, and all types of muscle fibres) – was called acetylcholinesterase, as acetylcholine is preferentially hydrolyzed. The enzyme in plasma was termed 'butyrylcholinesterase', as it preferentially hydrolyzed butyrylcholine and was formerly more popularly known as pseudocholinesterase. The roles of butyrylcholinesterase and that of acetylcholinesterase in erythrocytes and plasma are not known with any certainty to date, and individuals who do not possess butyrylcholinesterase lead normal lives until they are exposed to succinylcholine.

Butyrylcholinesterase is involved in the hydrolysis of many therapeutic agents and, together with acetylcholinesterase in the blood, it acts as a site for phosphorylation by organophosphates, thus serving as scavengers [8]. Scavenging reduces the amount of organophosphate available for toxic effects at vulnerable targets. For both acetylcholinesterase and butyrylcholinesterase, several functions have been proposed that are not directly related to synaptic transmission, e.g. the regulation of protein–protein interactions during neurite outgrowth and synapse formation, the modulation of cell movements, and cell proliferation [9].

A variety of methods are now available for cholinesterase assays based predominantly on the measurement of the rate of hydrolysis of an ester catalysed by cholinesterase. However, as the 'normal range' is characteristic for each substrate and the rate of hydrolysis is temperature- and pH-dependent, confusion has arisen in the interpretation of estimations when the exacting practice of recording the substrate, pH and temperature have been omitted [1]. There are several reasons for the confusion associated with cholinesterase estimations in relation to exposure to anticholinesterases (e.g. organophosphates and carbamates). There are many causes of decreased activity of cholinesterases that are not related to exposure to anticholinesterases: genetic, physiological (age, gender, pregnancy, etc.), iatrogenic (therapeutic agents), disease states, exposure to smoke fumes and, in some instances, of uncertain origin [10–12]. There are suggestions that dietary factors can influence cholinesterase concentrations. Low concentrations of cholinesterases have been observed in malnutrition [13]. Further, with the increasing popularity of traditional medicines containing plants or plant extracts capable of lowering cholinesterase activity (e.g. solanine and chaconine in potatoes,

Correspondence to: Lakshman Karalliedde, Medical Toxicology Unit and National Poisons Centre (London), Guy's and St Thomas' NHS Trust, Avonley Road, London, SE14 5ER, UK. E-mail: Lakshman.Karalliedde@gstt.sthames.nhs.uk; Tel: +44 (0)207 771 5315/5202; Fax: +44 (0)207 771 5306

which are often used in African traditional medicines to treat human immunodeficiency virus infection; also huperazine A in Chinese folk medicine, which is three times more potent than physostigmine in inhibiting acetylcholinesterase), other causes of lowered cholinesterase concentrations need to be considered [14]. At an American Society of Anesthesiologists' annual meeting, an anaesthetist reported that butyrylcholinesterase was inhibited at about 1% of the glycoalkaloid dose needed to block acetylcholinesterase and 'that amount was detected in the blood of individuals who have consumed potatoes'. The US Department of Agriculture has documented cases of toxicity after potato consumption [15]. In addition, there is considerable interindividual variation, the coefficients obtained from plasma cholinesterase ranging from 15 to 25%, while for erythrocyte acetylcholinesterase the range was rather less at 10–15%. The above findings are compounded by the observation that successive monthly measurements – or successive daily measurements – in healthy individuals revealed intraindividual variations >20%, which on occasions reached 40% [16].

Raised concentrations of acetylcholinesterase have been detected in the presence of neural defects in early pregnancy [17]. They may prove reliable indicators of changes developing during the course of the disease multiple myeloma, e.g. remission fulfilment [18]. The mean butyrylcholinesterase concentrations of chronic spinal pain patients were significantly higher than the mean concentrations in normal control volunteers [19]. The observation that concentrations decreased significantly during anaesthesia was considered to be of some value in the treatment of chronic pain. Acetylcholinesterase determinations made after myocardial infarction have enabled classification of such patients into four groups with defined prognostic values [20], and assays prior to discharge of such patients has been recommended. Some correlations have been reported during the course of tetanus infections [21] and following liver transplantation [1]. Aldridge, in 1953, provided reasons for the different sensitivities of butyrylcholinesterase and erythrocyte acetylcholinesterase for organophosphate inhibitors [22].

Since assays for blood cholinesterase activity have become relatively simple procedures, they continue to be used to assess the extent of human exposure to pesticides. The role of such assays in diagnosis is more accepted than their role in estimating severity or prognosis and recovery. In industrial or occupational exposures, the cholinesterase assay has a clear advantage over estimations of atmospheric contamination of pesticides because estimations of contamination and of rates of inhalation are difficult in agricultural or farming conditions. Furthermore, the equipment for such measurements is much less freely available. Moreover, atmospheric analysis does not take into consideration absorption by other routes such as the skin, a real hazard among those who mix and apply pesticides. Cholinesterase assays have to some extent ensured that prescribed safety precautions during manufacture and application have been implemented [23]. Clear correlations between succinylcholine apnoea and butyrylcholinesterase assays are established and have been found to be very useful in the diagnosis of prolonged response to drugs used during anaesthesia. However, the same cannot be said for exposure to anticholinesterases. Among the puzzles surrounding the widely used organophosphates is the persistent failure to correlate biochemical findings with the severity of the disease following their exposure [24]. In 1946, Comroe and colleagues observed that di-isopropyl-fluorophosphate – which is an organophosphate capable of lowering butyrylcholinesterase and erytrocyte acetylcholinesterase activity to zero – afforded less symptomatic relief to patients suffering from myasthenia gravis than neostigmine, which was a less potent inhibitor of cholinesterase. They agreed with the 'fallacy' of relating pharmacological and therapeutic effects of anticholinesterases to the cholinesterase-lowering activity, which had previously been suggested [25]. However, it is now known that the solubility properties of neostigmine and di-isopropyl-fluorophosphate may have contributed to Comroe and colleagues' observations. In 1963, Goldberg and colleagues [26], studying the carbamate compound 10854, observed that effects on animal behaviour occurred after doses that were considerably lower than those that produced overt symptoms characteristic of anticholinesterase action. Grob [27], reporting on the effects of sarin in human beings, commented that local ocular and respiratory manifestations of nerve gas poisoning may occur without any inhibition of plasma or erythrocyte cholinesterase. However, he noted that systemic manifestations were invariably accompanied by depression of activity of these enzymes but varied depending on the form of exposure. The plasma and erythrocyte cholinesterase concentrations at the time of onset of symptoms following a single exposure were depressed approximately by 60 and 50% of initial activity following inhalation, to 35 and 25% following ingestion of liquid, and by 15 and 10% after percutaneous exposure. He went on to state that following repeated exposures to nerve gases, there was no predictable correlation between the onset of symptoms and the precise levels of cholinesterase activity in both plasma and erythrocytes, except that both were depressed considerably to values below normal.

A review by Karczmar in 1984 [28] suggested that anticholinesterases might directly affect a

second messenger as well as a transmitter system other than the cholinergic system. Monnet-Tschudi and colleagues [29], studying the toxic effects of the organophosphates chlorpyrifos and parathion and their oxide derivatives on foetal rat brain cell cultures, suggested that toxic effects and acetylcholinesterase inhibition were unrelated. Murata and colleagues [30] investigated the victims of the Tokyo subway attack 6–8 months following the event and observed the persistence of asymptomatic sequelae to sarin exposure in the higher and visual nervous systems that exceeded the turnover period of cholinesterase. This latter observation arouses a certain degree of concern. Similarly, there is debate at present about the consequences of exposure to pesticides (organophosphates in particular) during the neonatal period and early infancy [31]. Vulnerability of a developing nervous system to their action particularly during periods of intense activity such as the 'rapid brain growth spurt' is uncertain but arouses an intense and often emotive response. Further, the phenomenon referred to as 'imprinting' [32] adds to concerns. This suggests that exposure to a wide range of chemicals during the neonatal period produces unusual responses following further exposure to similar agents in adult life.

Thus, doubts expressed after good research in the late 1940s and 1950s continue to trouble us today despite some correlations reported with symptomatology during the acute cholinergic phase, development of the intermediate syndrome [33] and for weaning from ventilatory care [34]. It is useful to recall the words of Ladell [35]:

> the degree of dysfunction in a cholinergic junction is not linearly dependent upon the amount of cholinesterases present for there are considerable reserves of enzyme at all sites and the amount required for efficient functioning is small in comparison with the total amount required. The percentage depression would be the same at all sites and in all tissues within the body – blood, brain, autonomic ganglia, muscles and glands – if the relative concentration of cholinesterase to inhibitor was the same everywhere after poisoning, which requires rapid penetration to all sites.

The most important consideration for the reluctance to accept cholinesterase estimations as the sole measurement of value in anticholinesterase exposure is that clearly one cannot attribute all aspects of ill-health following such exposures only to inhibition of cholinesterases. Certainly, the development of organophosphate-induced delayed polyneuropathy is not associated with cholinesterase depression [36]. The relatively lesser known applications of cholinesterase measurements in clinical medicine, e.g. myocardial infarction, the detection of neural birth defects,

multiple myeloma, and the management of chronic pain and tetanus, certainly merit further study. The role of cholinesterase estimations in anaesthetic practice will continue to be valuable, for these enzymes are necessary for the hydrolysis not only of succinylcholine and mivacurium, but also for that of diamorphine, cocaine, esmolol, remifentanil, procaine and related local analgesics. Cholinesterase concentrations may also be depressed by a wide range of therapeutic agents that may be prescribed for subjects presenting for anaesthesia [37].

Finally, whatever the limitations, cholinesterase estimations remain the only useful biochemical tool in anticholinesterase exposure at present. However, there are doubts after nearly four decades that it can be described as a 'trusted tool'. Therefore, critical assessment is needed soon and, if necessary, the search for a more sensitive and reliable biochemical marker for anticholinesterase exposure (in particular organophosphate exposure) should be accelerated. Critical evaluation of alternatives such as carboxylesterase estimations and plasma β-G levels is also required with some sense of urgency. Perhaps hair analysis, which has been successfully developed to detect long-term exposure to drugs of abuse, should also be considered for occupational exposures.

L. Karalliedde
Medical Toxicology Unit and
National Poisons Centre (London)
Guy's and St Thomas' NHS Trust
London, UK

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