LABORATORY EXAMINATION
IN NERVE AGENT INTOXICATION

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Summary: Diagnosis of nerve agent intoxication is based on anamnestic data, clinical signs and laboratory examination. For acute poisoning, cholinesterase activity in the blood (erythrocyte AChE, plasma/serum BuChE) is sensitive, simple and most frequent laboratory examination performed in biochemical laboratories. Specialized examinations to precise treatment (reactivation test) or to make retrospective diagnosis (fluoride induced reactivation etc.) can be conducted. Other sophisticated methods are available, too.

Introduction

Chemical weapons (CW) belong to the weapons of mass destruction. Their development, production, use etc. are prohibited and controlled by the internatonal coonvention (Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and on their Destruction, CWC) (16). After ratification with sufficient number of the State Parties, CWC entered into force (29 April 1997) and at present, 188 State Parties are involved including Czech Republic. In the Czech Republic, the State Institute for Nuclear Security (Státní ústav pro jadernou bezpečnost, SÚJB), was established as the executive and control organ responsible for implementation of the Convention in the Czech Republic. Simultaneously, this organ licensed laboratories for ability to work/handle with highly toxic chemicals such as chemical warfare agents (CWA). Without the license it is impossible to work/manipulate with CWA (e.g. sarin, soman etc.) and this condition is limiting factor for analysis of these agents. Though the use of CWA is in general prohibited by the Convention, it is not excluded their misuse either in military or terroristic actions (57).

The group of nerve agents is the most important group of CWA. The stocks of these compounds (weaponized or stored) in the Albania, India, South Korea, United States and Russia (officially declared) represent about 69,429 tons (53); it is quite clear that the paper is focused to these agents.

Laboratory diagnostics following poisoning with nerve agents is focused mostly to:
– determination of the mother compound or metabolites; examination is specific for such compound but it is lim-

ited to the acute stage of intoxication; usually it is not very sensitive, depending on the dose administered;
– determination of changes caused by the agent (group of agents) in question; it is mostly less specific indicating more group of agents. However, it is sensitive and detectable long time interval after the intoxication.

Both determinations are realized mostly in the blood (erythrocytes/serum/plasma) but some other materials are not excluded (e.g. tissues such as the brain, muscle, saliva, cerebrospinal fluid etc.). It can be used for forensic purposes, too. The problem is determination/information on the normal values in biological sample. For further studies, it can be recommended the monography dealing with many aspects of CWA edited by Professor Gupta (25).

Nerve agents

Nerve agents belong to the highly toxic agents; they are the most important group from CWA having high toxicity, rapid action at all routes of administration including inhalation and percutaneous exposure. They can be divided into two main groups, G-agents and V-agents; between them, group of agents combining in their structures G an V group is observed, called GV (GP) agents. It can be pointed out that compounds of the same basic structure (organophosphates, OP) are used in industry, veterinary or human medicine and in agriculture, e.g.. Metathion, Malathion, Actellic, Dichlorvos (DDVP), Paraoxon, Parathion, In-stop etc. (Fig. 1)

In the CWC, examples of nerve agents containing general structure are given (Schedule 1, CWC) (16):

O-Alkyl ($\leq C_{10}$, incl. cycloalkyl) alkyl (Me, Et, n-Pro or i-Pro) phosphonofluoridates, e.g. sarin, soman;
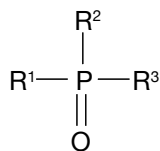
Fig. 1: General structure of organophosphates: R^{1-2} are hydrogen, alkyl (including cyclic), aryl and others, alkoxy, alkylthio and amino groups. R^3 is a dissociable group, e.g. halogens, cyano, alkylthio group, rest of inorganic or organic acid. More about the chemistry of organophosphates see e.g. Fest and Schmidt (23); modern trends in the development of nerve agents was described by Halámek and Kobliha (27)

O-Alkyl ($\leq C_{10}$, incl. cycloalkyl) N,N-dialkyl (Me, Et, n-Pro or i-Pro) phosphoramidocyanidates, e.g. tabun;

O-Alkyl ($\leq C_{10}$, incl. cycloalkyl) S-2-dialkyl (Me, Et, n-Pro or i-Pro)-aminoethyl alkyl (Me, Et, n-Pro or i-Pro) phosphonothiolates and corresponding alkylated or protonated salts, e.g. VX.

Thus, all these chemicals having above mentioned structure are under control of the CWC.

Mechanism of their action, symptoms, principles of diagnosis and treatment for OP and nerve agents are very similar. Structures of some nerve agents and OP are shown in Fig. 2.

Pharmacodynamics of nerve agents

Irreversible acetylcholinesterase (AChE, EC 3.1.1.7) inhibition at cholinergic synapses is basic toxicodynamic action of nerve agents. Increased level of the neuromediator acetylcholine causes symptomas observed in the course of poisoning. These symptoms are muscarinic, nicotinic and central as described in detail many times (3, 5, 14, 25, 36, 44, 58 etc.). However, before action at cholinergic synapses, nerve agent is penetrating into the blood, and, by this transport system is distributed to the target organs – peripheral and central nervous system. During penetration nerve agents react with the blood cholinesterases – erythrocyte AChE and plasma butyrylcholinesterase (BuChE, EC 3.1.1.8). The part of the agent bound to cholinesterases is excluded from toxic action. To the targets, only a part of the dose administered is penetrating and inhibiting AChE at cholinergic synapses. Schematic representation of interaction of AChE with nerve agent/organophosphate is shown in Fig. 3.

AChE (E) is reacting with nerve agent (P) to an intermediate complex (EP) and then to phosphorylated (phosphonylated) complex EP1. The complex EP1 is reactivatable by cholinesterase reactivators (R). EP1 complex is able in some cases to be changed to non-reactivatable complex (EP2) – reactivators are not effective. This reaction (EP1 → EP2) is called aging of inhibited AChE and, typically, it is observed for interaction of AChE with sarin and soman (Fig. 4). Relevant rate constants ($k_{+1,-1,+2,+3,+4}$ and k_a) for the reactions are indicated. The complex EP is not easily detectable, EP1 and EP2 complexes can be determined. Their distinction is difficult. Non-cholinergic effects are not specific and they are observed in long term intervals (hours–days) following intoxication (3, 5, 32, 36, 44).

Spontaneous recovery of AChE activity (reactivation) is long lasting as well as the synthesis of AChE de novo (3, 5, 36, 44, 58, 59). Reaction of AChE and nerve agents is of
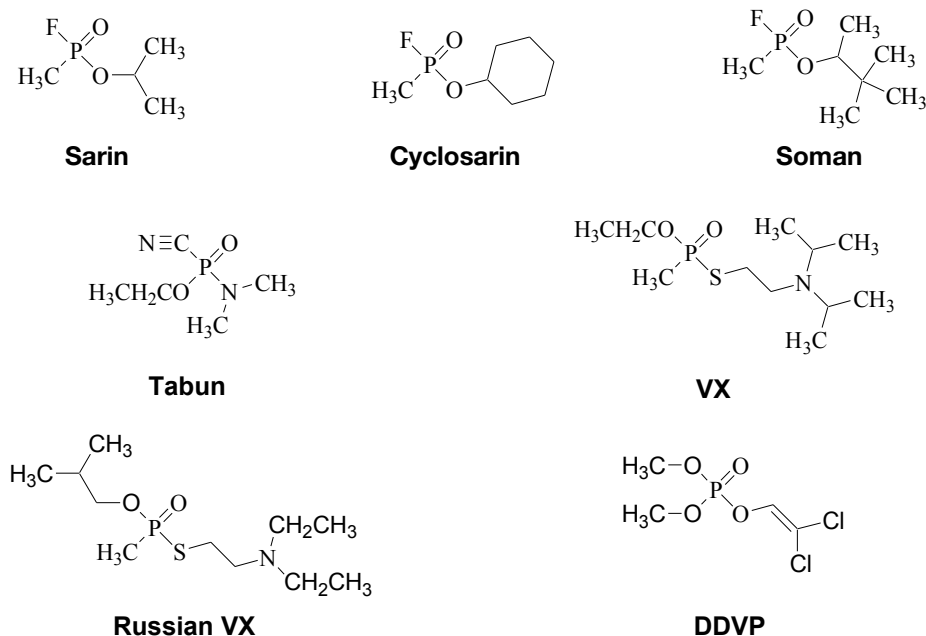

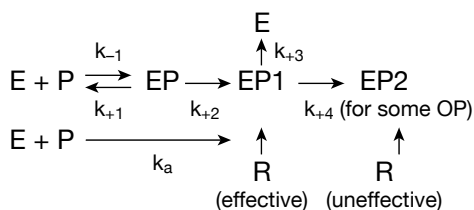**Fig. 2:** Structural formulae of some nerve agents and DDVP

Fig. 3: Schematic representation of interaction between AChE (E) and nerve agent/organophosphate (P)

classic inhibition kinetics: reactions important for laboratory diagnosis are shown in Fig. 3.

The effect of reactivators is limited by the rate of aging process; it is chemical reaction when inhibited AChE is changed to be resistant to the effect of reactivators. The rate of this reaction is dependent on the time and the structure of inhibitor. The half-lives of aging for soman are reported to be in minutes; for sarin, the half-life is about 10 hours and AChE inhibited with VX is dealkylated within 24 and more hours (e.g. 5, 36). This fact is limiting factor for the treatment using reactivators. For OP insecticides, this reaction is not practically important (they are not dealkylated) (Fig. 4).

Pharmacodynamics of nerve agent can be described by the simple scheme containing following steps – penetration (resorption) through biological barriers (dependent of the route of administration), transport and distribution by the transport system (blood) to sites of metabolic and toxic effect (Fig. 5).

In the scheme, possible material for laboratory diagnosis is indicated. During these reactions, the losses originated caused by detoxification, binding of the agent to enzymes (cholinesterases and other hydrolases) and proteins and thus, the real amount of the agent penetrating to the target sites (cholinergic synapses at the central and peripheral nervous system) reaches to a small fraction of the dose administered (10% and less). Detoxification of nerve agents and organophosphates was described in detail by Jokanovič (29). The binding of nerve agent to cholinesterases is important and advantageous for diagnostic purposes: blood cholinesterases are easily accessible for laboratory examination.

AChE a BuChE differences

AChE and BuChE are similar enzymes differing in their localisation, enzymatic properties, sequence of amino acids and physiological function (3, 5, 13, 17, 37, 45, 52, 61). AChE is observed at cholinergic synapses, erythrocytes and BuChE is contained mostly in plasma/serum and liver. AChE and BuChE have different sensitivity to substrates and inhibitors; inhibition by substrate is typical for AChE. The function of AChE is splitting neuromediator acetylcholine at cholinergic synapses; its function in erythrocytes is not yet known. The function of BuChE is not known, it can play a complementary role in cholinergic transmission or it is involved in non-specific detoxification reactions and neurological diseases (5, 17,18, 29).

A qualitative difference between the BuChE of suxamethonium sensitive individuals and that of other patients were described in detail by Whittaker (60). The biosynthesis of BuChE is controlled by two allelic genes, E^u_1 and E^a_1. Individuals with the combination $E^u_1 E^u_1$ are homozygotes with normal BuChE activity; a combination of $E^a_1 E^u_1$ (heterozygotes) and $E^a_1 E^a_1$ (homozygotes) resulted in diminished BuChE activity. The presence of a silent gene (E^s_1) was also proposed and a fourth gene controlling biosynthesis of BuChE (fluoride resistant, E^f_1) was recognized; the hypothesis was in general established by family studies. When BuChE is genetically changed to lower activity, all of drugs containing ester bond (succinylcholine, some local anaesthetics) are not hydrolysed by BuChE. Thus, the dose of
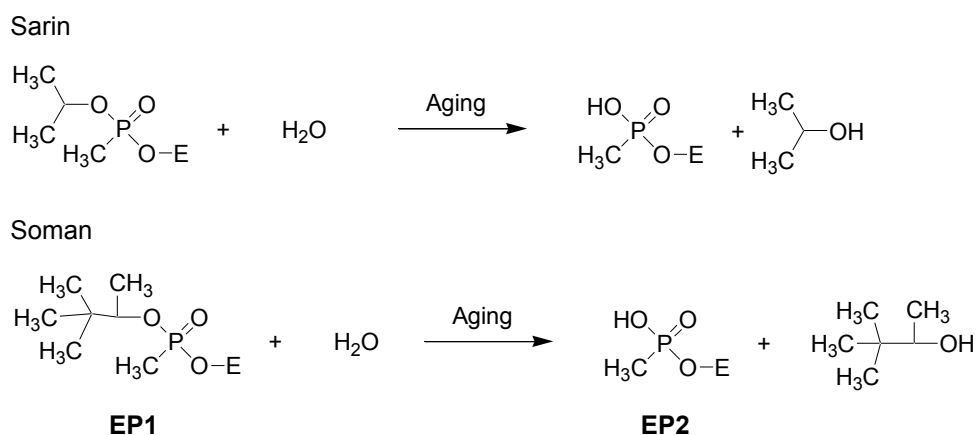

Fig. 4: Demonstration of aging for sarin and soman. It is the change of reactivatable complex (EP1) to not reactivatable complex (EP2) and forming relevant alcohol
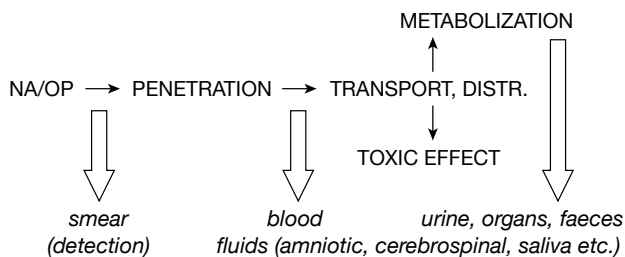
METABOLIZATION

NA/OP → PENETRATION → TRANSPORT, DISTR.

TOXIC EFFECT

smear
(detection)

blood
fluids (amniotic, cerebrospinal, saliva etc.)

urine, organs, faeces

Fig. 5: Importants steps for toxicodynamics (material for laboratory diagnostics ante or post mortem)

these drugs adminitered to the patient can be relatively high and some complications (e.g. succinyl choline apnoea) can be observed.

Diagnosis

Anamnestic data are very important for diagnosis of OP/nerve agent poisoning especially in connection with possible terroristic attack. Detection or identification of the agent is also very important, simultaneously with the clinical status of poisoned patiens and laboratory examination. Detection of nerve agent is possible using special equipment. Clinical signs such as miosis and failure of accomodation, salivation, lacrimation and sweating, breath difficulties, nausea and vomiting and, later on, fasciculations and convulsions are important clinical signs.

The most important diagnostic test is determination of cholinesterase activity in the blood (erythrocyte AChE or plasma/serum BuChE) (5, 31, 56, 57). Specialized examinations mostly needs more sophisticated methods (11, 12).

From practical point of view, in nerve agent intoxication, erythrocyte AChE activity is determined. It correlates with symptoms of poisoning and with AChE activity/inhibition not only in the peripheral target organs (diaphragm), inhibition is very similar to that observed in the central nervous system (ponto medullar area of the brain). Therefore, AChE

activity in the red blood cells corresponds with pharmacodynamics. It is more valid for nerve agents; for OP insecticides, the correlation is not so closed (5, 55, 58).

Table 1 shows changes in erythrocyte and brain (pontomedullar area) AChE activity following intoxication with nerve agents (sarin, soman, VX). When the blood activity is decreased to 70% of normal values (without any symptoms), it is indication for exclusion of the worker from contact with nerve agent. At this inhibition level, no symptoms are observed. When the activity is decreased to 40–70% of normal values, muscarinic symptoms are observed; further decrease to 15–30% correlates with fasciculations and convulsions, and, at the time of death, the activity determined is cca 5% and less. Moreover, the decrease of AChE activity in the erythrocytes copied the activity changes in the target organs. However, it is necessary to point out that activity of erythrocyte AChE and plasma BuChE varied individually (more for BuChE), and therefore, individual normal activity in workers with nerve agents is recommended.

From non-specific examinations, leukocytosis and an increase of aminotransferases are detected. For final diagnosis, reactivation test is very useful. The principle of the test is double determination of cholinesterase activity – the first one without reactivator, the second one with reactivator. The direct determination of the nerve agent (or metabolites) is possible but it is limited to short period after the exposure. Retrospective diagnosis based on specialized examination is also valuable (as it was demonstrated in sarin victims in Japan) but it requires sophisticated approach (usually GC with MS) (42).

Smear and detection

Smear and detection of contaminated material is possible but these methods are not suitable for clinical laboratory, it is purely chemical approach.

Free nerve agent

Detection of the free agent is possible in the blood/plasma but the detection is timely limited to hours after

Tab. 1: Symptoms of nerve agent intoxication and AChE activity changes in erythrocytes (man, dog) and in erythrocytes and brain (pontomedullar area, PM) in rats and mice. Table elaborated using data Bajgar et al. (6, 7); Bajgar (5); Capacio et al. (14); Jun et al. (30)

symptoms	human (blood)	dog (blood)	rats (blood/PM)	mice (blood/PM)
control	100	100	100	100
asymptomatic (professional medicine limit)	70	65–72	75/80	65/75
salivation	50–60	51–68	66/73	60/68
fasciculations	20–30	25–38	27/36	25–29
convulsions	10–20	12–18	13/15	10/11
death	0–5	0–3	6–9/4–13	0–3/5–10

the exposure. Disadvantage is the necessity of calibration requiring original agent not known at the beginning of intoxication. This situation is similar for metabolites.

Metabolites

In the case of sarin, soman and cyclosarin exposure, these agents are metabolized through their relevant acids – isopropylmethyl-, pinacolylmethyl- a cyclohexylmethyl-phosphonic acids, finally forming methylphosphonic acid MPA):

$$H_3C - \overset{\overset{\displaystyle O}{\displaystyle \|}}{\underset{\underset{\displaystyle OH}{\displaystyle |}}{P}} - OH$$
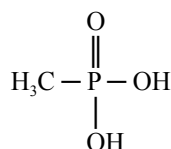
In case of VX exposure, diisopropyl aminoethyl methyl sulphate or MPA can be detected. Metabolites mentioned (mostly MPA) can be detected in urine, too. The methods for detection are mostly combination of GC-MS, LC-MS or GC-FPD and other combinations (9, 20, 51, 54). In case of some OP (e.g. parathion, paraoxon) is possible to detect their metabolite in urine (p-nitrophenol) using simple spectrophotometric method (4, 22).

AChE activity in erythrocytes

Determination of AChE activity can be performed using different methods based on various principles: in each case, substrate (either natural – acetylcholine or artificial – e.g. acetylthiocholine) is added to biological sample and unhydrolysed substrate or reaction products are determined by different methods: spectrophotometric, pH changes indicators, colorimetric, polarographic, fluorimetric, radiochemical, enzymatic and others. The determination can be performed continually or discontinually, it is possible to increase the specificity using selective AChE or BuChE inhibtors (review see e.g. 4, 5, 14, 42, 43).

The most common method is original Ellman's method (21) and its modifications. It is based on hydrolysis of the thiocholine substrates (acetyl- and butyrylthiocholine or others). After enzymatic hydrolysis, the relevant acid and thiocholine are released and SH-group of thiocholine is detected using 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) forming 5-mercapto-2-nitrobenzoate anion determined spectrophotometrically at 412 nm. Sometimes this method is used with specific inhibitors and there are many modifications described in the literature. This method is in good correlation with other methods. It is sufficiently specific and sensitive and it is used for different purposes in many laboratories around the world. It is very useful in monitoring of workers exposed to nerve agents or OP; evaluation and 40 years experience with this determination including suspect intoxications was described by Jun et al. (30). The disadvantage of this method is that the method is not used routinely in clinical laboratories. Theoretically, it would be easily per-

formed in the clinical biochemical laboratory by the change of substrate (not butyrylthiocholine but acetylthiocholine) or by the use of specific BuChE inhibitors. It is possible to determine AChE activity in the whole blood (3, 26); it is represented by 90% of AChE and 10% of BuChE (human) and 29% of BuChE and 71% of AChE (rat) (3). Advantage of this method is sampling – the blood is obtained from finger tip (50 µl) and it is not separated to erythrocytes and plasma by centrifugation (4, 5). The determination of individual molecular forms of AChE or BuChE is also possible using separation techniques (electrophoresis, ultracentrifugation etc.) – high molecular forms are more sensitive to nerve agents than low molecular forms (3). It is used more frequently for OP insecticides.

The expression of activity is important for comparison of different results described by various laboratories. It is expressed as µmoles of substrate hydrolyzed per time (minute) by defined volume (plasma/serum) or weight of the material (wet, dry, content of nitrogen etc.). Expression in Units is also possible. In the clinical laboratory, catals/l are used for the expression of enzymatic activity (kat/l) – it is 1 mol of substrate hydrolyzed per sec/liter or kg.

Reference values: normal AChE aktivity in erythrocytes or in the whole blood varies about 110.2 (±11.2) µkat/l and 133.3 (±14.5) µkat/l; optimal choice seems to be determination of individual activity before possible exposure or before work with nerve agents. This examination in these cases is obligatory.

BuChE activity in serum/plasma

Determination of cholinesterase activity is based on similar principles as those described for AChE (reviews see e.g. 3, 4, 5, 14, 43). The most common method is mentioned Ellman's method (21) and its modifications. The determination of BuChE in the blood is the basic method for diagnosis and therapy monitoring for OP poisoning; however, it is necessary to be combined with clinical observation.

BuChE determination in the plasma or serum is routine examination in clinical biochemistry. It is used more frequently than that of AChE in the red blood cells. BuChE decrease indicates either a diminishment of the enzyme synthesis or a decrease in the number of production cells in the liver (4); some drugs also influence the plasma BuChE activity. A special case of diminished BuChE activity is the hereditary affected presence of atypical variants of BuChE (59). BuChE activity can be affected by different drugs and diseases – except OP/nerve agents and carbamates there are hormones (e.g. peroral anticoncepion), some heavy metals, carbon disulphide; BuChE can be changed in gravidity, antitumor therapy, in some psychiatric and neurological diseases, γ-irradiation, liver diseases, myocardial infarction (2–5, 18, 37).

Reference values: in plasma/serum, the activities are reported in the range from 88.7 to 215.3 µkat/l. Normal values for plasma BuChE after more than 45 years experience were

determined to be 150.5 (±31.3) µkat/l; individual activity (at the first examination) is recommended. It is possible to separate individual isoenzymes of BuChE, however, there arises a question of separation medium (agar, polyacrylamide, etc.) and the method of quantitative evaluation of the activity of these isoenzymes (3, 4, 30, 41, 47, 58).

Reactivation test

The principle of the test is double determination of cholinesterase activity in pooled blood sample. The first determination is without adding of the reactivator and represents normal activity; if it is decreased, it is indication of exposure to nerve agents. According to the degree of inhibition, the symptoms and steps of the poisoning can be derived. The second determination in the presence of reactivator shows the response of inhibited cholinesterase to the oxime added; usually, it is higher than that without reactivator. The increase indicates that the enzyme can be reactivated (it is EP1); if the enzyme is unreactivatable, it indicates that the enzyme is in unreactivatable step (EP2) and the activity of the second sample will be the same or lower than the first one. The level of reactivation could be helpful in differential diagnosis – what type of nerve agent was used: in case of soman, zero or very low reactivatability is observed. Modere increase indicates intoxication with sarin, and, high reactivatability suggests VX intoxication (Table 2).

It is possible to use different reactivators for second determination and – depending on the results – to choose the best reactivator for the treatment. Disadvantage of this method is non routine realization in clinical laboratory. This test was experimentally verified on dogs and rats in vivo and on human and dog blood in vitro (1–3, 5, 7).

Other markers

Generally, hydrolases and esterases and phosphatases (alkaline and acid) are sensitive to nerve agents but the sensitivity and specificity is not very high. Transaminases (AST, ALT) can be slightly elevated as well as leukocytes but there are also non specific changes. In acute intoxication, it is necessary to search blood oxygen (and CO_2 saturation) and pH of the blood and the levels of acid metabolites (possible acidosis) (4, 5, 14, 36).

Tab. 2: Results of the reactivation test for human, dog and rat erythrocyte AChE following inhibition by nerve agents in vitro (human) and in vivo (rats and dogs). Table elaborated using data of Bajgar (1–3, 5); Bajgar et al. (7)

Nerve agent	Erythrocyte AChE		
	human	dog	rat
soman	0–10	15 (10–24)	10 (5–15)
sarin	25–65	63 (50–78)	55 (41–72)
VX	55–90	87 (70–100)	80 (60–95)

Stresogennic parameters

It is mostly corticosterone; its level is elevated 3–8 hours after the exposure. The concentrations of c-AMP and c-GMP are also elevated at these intervals (3, 44). Stressogenic effects as well as behavioral changes are observed also following exposure to low level of nerve agents (32).

Fluoride induced reactivation

The method of cholinesterase determination in the blood indicates the decrease of activity only (i.e. the presence of inhibitor). It does not to identify the mother compound, and, when the inhibition is too low (10–20%, i.e. the rest of activity is about 80–90%), it is in the limit of sensitivity. It is not suitable for retrospective diagnosis. The method of fluoride-induced reactivation has not these disadvantages. The method was developed by TNO-Prins Maurits Laboratory, Rijswijk, the Netherlands; it is based on reactivation of phosphylated cholinesterase and carboxylesterase (CaE, EC 3.1.1.1) by fluoride ions. Treatment of the inhibited enzyme with fluoride ions can inverse the inhibition reaction yielding restored enzyme and a phosphofluoridate which is subsequently isolated and quantified by GC and phosphorus-specific or mass spectrometric detection (46). Important fact is the presence of CaE or BuChE in the material examined. Human (and monkey) plasma does not contain CaE but its BuChE concentration is relatively high [70–80 nM (10, 39)], much higher than the concentration of AChE in blood [ca. 3 nM (28)]. Plasma of laboratory animals, such as rats and guinea pigs, contains considerable concentrations of CaE in addition to the cholinesterases. The method allows partial identification of the OP whereas the lifetime of the phosphylated esterase (and consequently the retrospectivity of the method) is only limited by spontaneous reactivation, in vivo sequestration and aging. The rate of the latter process (aging) depends on the structure of the phosphyl moiety bound to the enzyme and on the type of esterase. Phosphylated CaEs generally do not age.

Based on this method for retrospective detection of exposure to OP, exposure of victims of the Tokyo incident to a nerve agent, probably sarin, could be established from analysis of their blood samples (46). Later on, this method was used for the study of the effect of low inhalation dose of soman (6).

Alternatively, assays based on mass spectrometry have recently been developed which will also diagnose on the basis of the aged phosphonylated BuChE (24). The method was also modified and improved (19, 38).

Combination of different appoaches was able to establish definitive diagnosis of sarin use in Tokyo subway (40, 49). Thus, fluoride induced reactivation of nerve agent-inhibited AChE is a reliable and retrospective method to establish nerve agent-exposure. It is limited to compounds that regenerated with fluoride ions.

Tab. 3: Comparison of different methods for diagnosis of nerve agent intoxication

method	diagnostic validity for poisoning	instrumen- tation	time	frequency of use	difficult- ness	demanding- ness
smear	for exposure	+ – +++	minutes	++	+	+
free + metab	acute	+++	hours	++	++	++
AChE ery/wh bl	acute	+	minutes	++	+	+
BuChE pl/se	acute	+	minutes	++++	+	+
react test	acute + treatment	+	hour	+	++	++
other	developed	++	minutes	+	+	+
stress mark	developed	++	hour	+	+	++
fluoride react	retrospective (low doses)	+++	hour	++	++	+++
enzym dig	retrospective	+++	hours	+	+++	+++
adducts	retrospective	+++	hours	+	++	++

Enzymatic digestion

Fluoride induced reactivation is limited to compound able to react to adequate manner. This disadvantage is eliminated by the method of enzymatic digestion. This is a novel and general procedure for diagnosis of exposure to OP, which surpasses the limitations of the fluoride reactivation method (48). It is based on the rapid isolation of BuChE from the plasma by the affinity chromatography, digestion with pepsin followed by liquid chromatography with the mass spectrometric analysis of phosphylated nonapeptides resulting after the digestion of inhibited BuChE with pepsin (15). The method can be applied for the detection of exposures to various OP pesticides and nerve agents including soman. This approach is very valuable and represents a new field for the improvement of diagnosis with nerve agents and OP. A comprehensive review of the methods for retrospective detection of exposure to toxic scheduled chemicals has been published by Noort et al. (43). Method allows to confirm retrospective exposure to different OP and nerve agents including soman. For review see also e.g. ref. 4, 12, 14, 42, 43, 56.

Adducts

New approach was described by Lockridge et al. (35). It is based on detection of OP/nerve agent adducts with tyrosine (from proteins in the blood), without binding to cholinesterases (8, 50). This method opens new possibilities of detection aspecially in case of OP having less inhibition efficacy to plasma BuChE (33).

Summarization of the methods shows Table 3.

Conclusions

From laboratory examinations used for diagnosis of nerve agennt poisoning, determination of AChE/BuChE activity in the blood remains as basic, sensitive and frequently used method important for acute poisoning. Other methods are available for specification of therapeutic countermeasures, low doses exposure or retrospective diagnosis.

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