Red Blood Cell Acetylcholinesterase and Cholinesterase Status: Important Tools for the Physician in a Nerve Agent Scenario

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ABSTRACT

The increasing threat of an attack with nerve agents calls for effective diagnostic and therapeutic preparedness. Inhibition of acetylcholinesterase (AChE) is regarded as the most important toxic mechanism of nerve agents and therefore should be an integral part of casualty diagnosis and treatment monitoring. Therapeutic strategies are directed to competitively antagonise overstimulation of muscarinic receptors by using atropine and to reactivate inhibited AChE by utilizing oximes. The later approach is crucial, especially within the neuromuscular synapse where atropine is ineffective, *since peripheral neuromuscular block eventually leads to respiratory failure. Accordingly, the emergency physician has to identify patients with nerve agent intoxication as early as possible to ensure effective oxime treatment. Together with clinical signs and symptoms, a significantly reduced red blood cell (RBC) AChE activity clearly indicates intoxication by a cholinesterase inhibitor and the necessity for oxime treatment. Hence, an early determination of this parameter is mandatory.*

During an international NBC-defence exercise (Precise Response 2006, Canada) anesthetized pigs were poisoned with sarin followed by treatment with atropine and oxime. A diagnostic group of the German Medical-Chemical Task Force drew blood samples and determined RBC-AChE with a portable test system (Test-mate®). Marked inhibition of AChE together with clinical signs and symptoms verified within a few minutes that the animals were intoxicated with a cholinesterase inhibitor. After administration of an HI-6 bolus a rapid increase in RBC-AChE activity could be recorded on site, indicating effectiveness of the oxime treatment. In addition, pig blood samples were sent to our laboratory in Munich and were reanalysed with an automated analysis system (Tecan RMP). Comparison of results showed that an almost identical course of the AChE activities was recorded by both systems.

In addition, the cholinesterase status (AChE and butyrylcholinesterase activities, inhibitory activity in plasma and reactivatabiltiy of AChE) was determined using the automated analysis system in Munich. This more comprehensive laboratory test system is mandatory for optimizing therapeutic drug monitoring and oxime treatment in a clinical setting. Oxime administration can be stopped when AChE is aged completely but has to be continued as long as poison is present in the body and reactivation is possible.

To aid the on-site physician in optimizing diagnosis and treatment, the fielded test system should be improved to allow for rapid determination of cholinesterase status in standard medical treatment facilities.

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1.0 INTRODUCTION

The terrorist use of Sarin and VX in Japan (1) showed the world that nerve agents are no longer restricted to chemical battlefields but are a pertinent threat for civilian and military populations. Effective therapeutic preparedness is mandatory. Inhibition of acetylcholinesterase (AChE, EC 3.1.1.7) is regarded as the primary mechanism for the acute toxicity of all organophosphorus compounds (OP), nerve agents as well as insecticide OP, and therefore should be an integral part of casualty diagnosis and treatment. Therapeutic strategies are directed to competitively antagonise overstimulation of muscarinic receptors by atropine and to reverse AChE inhibition by reactivation with oximes. The later approach is crucial within neuromuscular synapses where atropine fails, since peripheral neuromuscular block eventually leads to respiratory failure. A lot of research has been performed over the last several decades, leading to a better understanding of the individual reactions occurring during inhibition and in the post-inhibition phase (aging, spontaneous reactivation, oxime induced reactivation, formation of phosphoryloximes and their interactions; for review see (2)). It can be concluded that all of these reactions must be considered for the optimal treatment of intoxicated patients.

2.0 DEVELOPMENT OF AN OPTIMAL TREATMENT REGIMEN

Treatment of signs and symptoms due to overstimulation of muscarinic receptors: Typical signs and symptoms of cholinergic crisis develop in direct correlation to the degree of acetylcholinesterase inhibition (3). In severe poisoning, respiratory depression, bronchospasm, bronchosecretion and weakness of the respiratory muscles calls for immediate endotracheal suctioning and artificial ventilation. Generally, heart rates between 80 and 100 beats/min, absence of rales during auscultation and dryness of the skin (axilla) should be achieved by treatment with muscarinic receptor antagonists, e.g. atropine. Furthermore, frequent circulatory insufficiency may call for treatment with catecholamines, to increase and maintain blood pressure (i.e. arterial mean pressure above 60 mm Hg with systolic pressure exceeding 100 mm Hg) as well as heart rate. In the later phase of severe intoxication, rales might be a result of cardiac insufficiency or (aspiration) pneumonia which is accompanied by elevated temperature and sweating, thus preventing the use of these parameters as clear indicators of atropine demand. In conclusion, in the acute phase of intoxication, atropine dosing should follow a protocol guaranteeing an early sufficient atropinization. Such protocols are proposed in modern literature (4;5), recommending a starting dose of 2 mg i.v. followed by an observation period of 5 min. If there is no effect, this dose may be doubled every 5-10 minutes until muscarinic symptoms subside. With this regimen after the 5^{th} dose, 62 mg of atropine would have been administered, an amount that should be sufficient. For the ongoing treatment, especially at intensive care units, the whole clinical picture has to be considered and dosing of atropine should be performed cautiously (e.g. 1-4 mg/h) to avoid adverse effects.

Reactivation of acetylcholinesterase: As atropine is not able to counteract signs and symptoms caused by the overstimulation of nicotinic receptors, especially at neuromuscular endplates, the concept of reactivation of inhibited AChE was developed (6) and pralidoxime (7) and obidoxime (8) were introduced fifty years ago into clinical therapy. Since the sixties, therapeutic effective concentration of 4 µg/ml oxime has been suggested in text books. This recommendation is based on a study in cats, intoxicated by a sarin analogue and then treated with pralidoxime. The result from this investigation " ... plasma concentrations of about 2×10^{-5} M (4 µg/ml) were needed to counteract neuromuscular block, bradycardia, hypotension and respiratory failure ..." (9) was extrapolated without critical evaluation for treatment of any OP-type-poisoning, using any oxime, and in any species. However, various organophosphorus compounds show quite different properties in reacting with AChE (10) and the reactivatability is dependent on both the properties of the OP-AChE complexes and of the oxime used (2;11). Moreover, enormous differences between species have to be considered (12). Based on reaction constants derived from experiments with human RBC-AChE, reasonable plasma concentrations of oximes for reactivation of insecticide as well as nerve agent inhibited AChE can be calculated (2;11;13-19). Results indicate that AChE inhibited by most nerve agents and insecticide OP can be reactivated with obidoxime at a plasma concentration of approximately 10 µM. This plasma concentration can be adjusted by an i.v. bolus dose of 250 mg, followed by 750 mg obidoxime per day. In actual emergency situations using such a regimen was effective in reactivating AChE inhibited by insecticide OP (11;16;20). Comparably, Pawar (21) showed reduced morbidity and mortality in moderately severe insecticide OP poisoned patients treated with effective doses of pralidoxime (2 g loading dose, followed by 24 g/day). Hence, effective oximes should be administered in appropriate dosage as early as possible (e.g. by autoinjectors or i.v.) and effective concentrations should be maintained as long as reactivation is possible. The treatment period may be shortened to several hours in poisoning with OP where toxic concentrations persist only for a very short time in the body (e.g. sarin) or when OP-AChE-complexes age quickly (e.g. soman). In contrast, in poisoning with persisting nerve agents (e.g. VX) or after ingestion of huge amounts of the poison as frequently found in suicide poisoning, administration of oximes may be necessary for several days.

3.0 MONITORING OF THE CHOLINESTERASE-STATUS

Assessment of therapeutic effectiveness in OP poisoned patients is difficult due to a wide variety of therapeutic measures taken at intensive care units (e.g. artificial ventilation, sedation). To monitor the course of poisoning in OP poisoned patients, a laboratory test system, called cholinesterase-status was developed (16;22) and used in intensive care units (Figure 1) (11;20;23;24). The cholinesterase status is comprised of:

Red blood cell (RBC)-AChE activity: Blood is immediately diluted bed-side 1:100 (v/v) with ice-cold phosphate-buffer (0.1M, pH 7.4; 0.03% Triton X-100). AChE activity is determined according to a modified Ellman method (22).

Reactivatability of RBC-AChE activity is assessed by incubation of diluted blood samples from the patients with 0.1 mM obidoxime at 37°C for 30 min (22).

Inhibitory material, indicating presence of active poison in the body without its exact identification: Plasma of patients is incubated with standardized RBC-AChE obtained from a healthy donor at 37˚C for 1 hr. Thereafter the RBC-AChE activity is measured according to the modified Ellman method (22).

Plasmacholinesterase (Pl-ChE; EC 3.1.1.8) activity is determined by the Ellman method with some minor modifications using 1.0 mM butyrylthiocholine as substrate (22).

Determination of the cholinesterase status is possible in our laboratory by using an automated system with a capacity to perform 600 assays per day (25;26).

Furthermore, for determination of RBC-AChE and Pl-ChE an improved portable device, appropriate for the use in the field was developed (27).

To demonstrate the properties of cholinesterase-status a case report of parathion poisoning is presented (compare (28;29)). This example resembles a course of poisoning that might be expected in percutaneous intoxication with a persisting OP, e.g. VX. A 45-year-old man had ingested about 100 ml of a parathion containing solution. On presentation to the emergency physician, the patient was unconscious with severe signs and symptoms of cholinergic crisis. After administration of 1.5 mg of atropine the patient was intubated and artificial ventilation was intiated. At the local hospital gastric lavage was performed and the patient stabilized. After treatment with 2 bolus doses of obidoxime together with an atropine infusion, the patient was transferred to the intensive care unit of the toxicological department of the 2nd Medical Clinic, Technical University, Munich. Here, the obidoxime regimen was started (250 mg i.v. bolus, followed by 750 mg/24 h). With this regimen, a therapeutic plasma level of about 10 µM obidoxime was maintained (Figure 1, upper panel, A). Due to the persisting poison load, only partial reactivation of RBC-AChE could be achieved over approximately 4 days. During this period, very little aging occurred (Figure 1, mid panel, B) and Pl-ChE remained completely inhibited. When the poison load significantly decreased on the 4th day following initiation of the obidoxime regimen, complete AChE reactivation was achieved. Furthermore, based on RBC-AChE activity, reactivatability and obidoxime plasma concentration, a paraoxon concentration necessary for such a degree of inhibition could be calculated (29) by using the respective reaction constants (17). The time course of this theoretical curve closely fit the actual time course of inhibitory activity as determined in the intoxicated patient (Figure 1, lower panel, C).

In cases of exposure to nerve agents, early determination of RBC-AChE is mandatory to confirm clinical diagnosis without sophisticated verification of the agent used. For this purpose, the Test Mate® was developed by EQM research (27). The proper function of the device was tested in an international NBC-defence exercise (Precise Response 2006, Canada). During the training scenario, a diagnostic group of the German Medical-Chemical Task Force entered a chemical agent environment for treatment of victims of a terrorist attack. An anesthetized pig intoxicated with sarin emulated a poisoned patient. On arrival, the pig (~ 20 kg) displayed typical clinical signs of cholinergic crisis; salivation, miosis and dyspnea. Marked inhibition of AChE verified intoxication with a cholinesterase inhibitor within a few minutes. After administration of the oxime HI-6 (260 mg) in combination with atropine (0.6 mg) (bolus dose), a rapid increase in RBC-AChE activity was recorded on site. This was clearly accompanied by prompt clinical improvement, indicating the effectiveness of the oxime treatment. To simulate the situation following rescue and decontamination, two additional pigs were intoxicated with sarin and treated at a field intensive care unit. Here, HI-6 or obidoxime were administered in combination with atropine. As observed in the field, the clinical situation improved after oxime treatment, however repetitive atropine doses were necessary to counteract hypersalivation. In one pig diazepam was also required due to convulsions. These animals were maintained under anesthetic for approximately 2 hours during which time, blood sampling was conducted. RBC-AChE activity was determined on-site to monitor the course of treatment. In addition, blood samples were sent to our laboratory in Munich and were reanalysed with an automated analysis system (Tecan RMP). Comparison of the results showed almost identical values independent of the analytic system used (Figure 2, upper panel).

4.0 INVESTIGATION OF NEUROMUSCULAR TRANSMISSION

Investigations on muscle strips of various species revealed that oximes were able to restore muscle force which was paralyzed by OP, however several therapeutic gaps (e.g. soman) still exist (30-36). Aside from an unknown direct oxime reaction, recovery of muscle force was mainly attributed to reactivation of inhibited muscle AChE. In a series of experiments on phrenic nerve-diaphragm-preparations of mice circumfused with paraoxon, it was shown that obidoxime induced restoration of muscle force and this was clearly accompanied by an increase in muscle AChE activity (36). Comparably, in patients with insecticide OP-poisoning, it was reported that low RBC-AChE activity (<10%) was associated with marked decrement-phenomena (23) (stimulation of a nerve with frequencies of 30-50 Hz and recording of compound action potentials), indicating severe disturbance of neuromuscular transmission and urgent requirement of artificial ventilation (37;38). At RBC-AChE activity of about 15-20% mainly decrement-increment-phenomena occurred (23). This type of response is typically found in moderate to severe poisoning (37;38) presumably also indicating the need for artificial ventilation. At RBC-AChE activity above 30% little disturbance of neuromuscular transmission could be detected (23). AChE is encoded on a single gene in mammalian species (39) and therefore a similar structure may be assumed to occur throughout the body (2;40;41). Consequently, RBC-AChE should have comparable functional properties to synaptic AChE and therefore may be used as surrogate parameter, reflecting the AChE status at the synaptic site. However, when RBC-AChE is aged completely, restoration takes several months (42) while synaptic AChE recovers faster (43;44). Therefore under such conditions, RBC-AChE can no longer be regarded as suitable parameter to indicate the end of the cholinergic crisis. As Pl-ChE may show quite different properties concerning inhibition by OP as well as reactivation of the inhibited enzyme by oximes

(2;45) this parameter has to be used with caution. Under such conditions, investigation of neuromuscular transmission may be of help. Especially when ventilators are in limited supply e.g. mass casualties intoxicated with rapid aging OP, investigation of neuromuscular transmission could guide the physician whether artificial ventilation is necessary.

5.0 RECOMMENDATION FOR DIAGNOSIS AND TREATMENT OF PATIENTS WITH POISONING BY ORGNOPHSPHORUS COMPOUNDS

Determination of RBC-AChE activity should be performed as early as possible to confirm diagnosis of poisoning by inhibitors of cholinesterase.

Atropine should be given according to clinical signs and symptoms using a regime which ensures fast atropinisation without overdosing, e.g. doubling the doses every 5 min after a starting dose with 2 mg.

Effective oximes should be administered at appropriate doses as early as possible to OP intoxicated patients.

The effects of oximes may be assessed using the cholinesterase-status, thus allowing optimal oxime treatment: oximes should be administered as long as reactivation is possible and inhibitory material is present in the patient. Investigation of the neuromuscular transmission should complete the monitoring system as objective clinical parameter. Especially when RBC-AChE activity is completely aged, improvement of neuromuscular transmission may be the decisive parameter to indicate the end of the cholinergic crisis. This item urgently needs further investigation.

To aid the on-site physician in optimizing diagnosis and treatment, the assays with the fielded test system should be further advanced to allow for rapid determination of the extended cholinesterase status in standard medical treatment facilities.

6.0 FIGURES

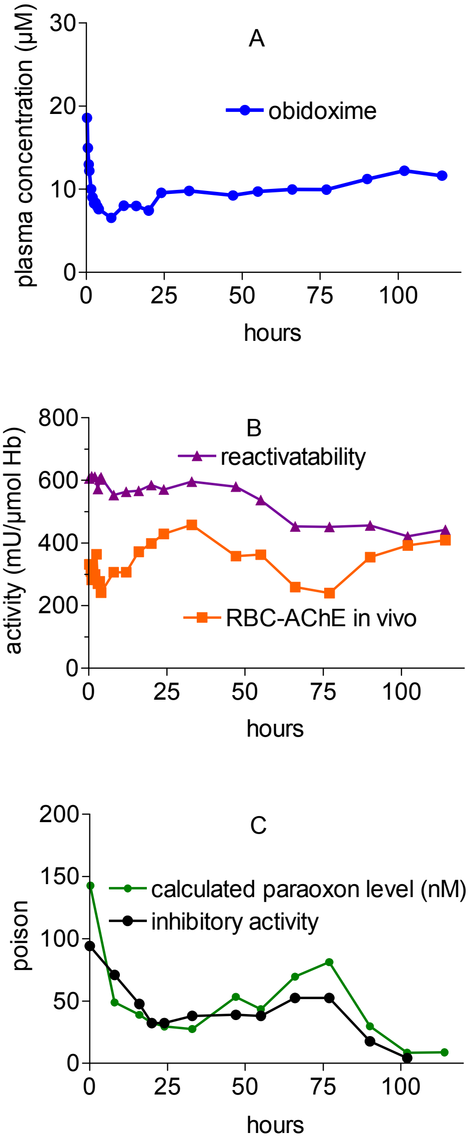

Figure 1. Cholinesterase-status of a patient with parathion poisoning.
Obidoxime plasma concentration was determined by HPLC according to Spoehrer at al. (46) (upper panel, A). RBC-AChE activity and reactivatability were determined according to a modified Ellman method (mid panel, B). Inhibitory activity (%) was estimated as AChE activity of donor RBCs incubated with the patient's plasma (lower panel, C, black line); and paraoxon (nM) calculated from RBC-AChE activity, reactivatability, obidoxime plasma concentration using the respective reaction constants (lower panel, C, green line) (29).

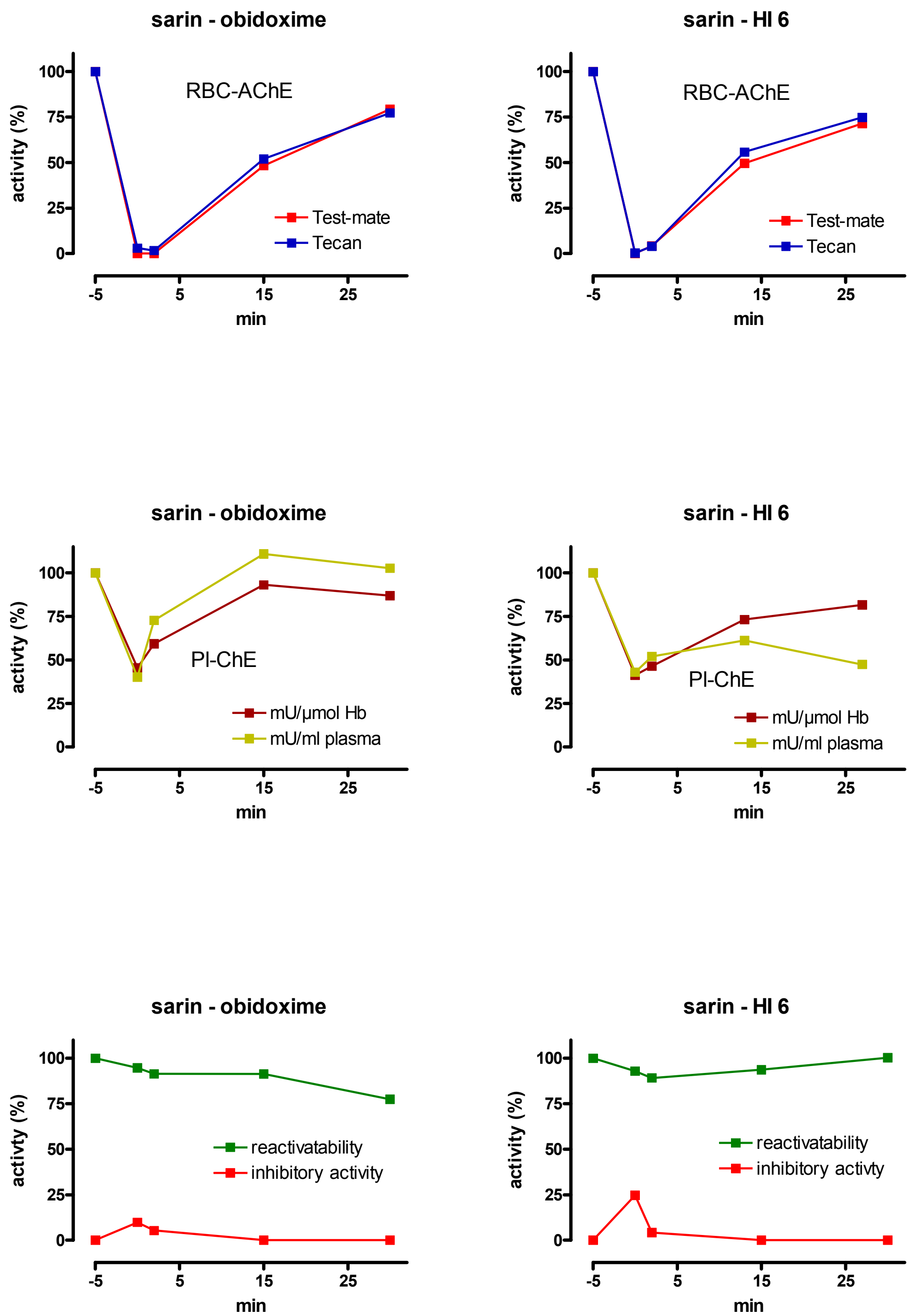

Figure 2. Cholinesterase activity, reactivability and inhibitory material determined in pig blood samples following sarin exposure. During the NBC-defence exercise Precise Response 2006 in Canada, anaesthetized pigs were intoxicated with sarin and treated with HI-6 or obidoxime. After the intoxication, RBC-AChE was determined on-site with the Test Mate® system and then with an automated system (Tecan) at the Bundeswehr Institute of Pharmacology and Toxicology in Munich. Additionally, Pl-ChE in blood (mU/µmol Hb) and plasma (mU/ml plasma) reactivatability and inhibitory material were determined in Munich.

Reference List

(1) Tu AT. Chemical terrorism: horrors in tokyo subway and matsumoto city. Fort Collins: Alaken Inc.; 2002.

(2) Eyer P. The role of oximes in the management of organophosphorus pesticide poisoning. Toxicol Rev 2003;22:165-90.

(3) Lotti M. Clinical toxicology of anticholinesterase agents in humans. In: Krieger RI, editor. Handbook of Pesticide Toxicology Second Edition.San Diego, San Francisco, New York, Boston, London, Sydney, Tokyo: Academic Press; 2001. p. 1043-85.

(4) Aaron CK, Howland MA. Insecticides: organophosphates and carbamates. In: Goldfrank LR, Flomenbaum NE, Lewin NA, Weisman RS, Howland MA, Hoffman RS, editors. Toxicologic Emergencies. Fifth ed. Norwalk, Connecticut: Appleton & Lange; 1994. p. 1105-16.

(5) Eddleston M, Buckley NA, Checketts H, Senarathna L, Mohamed F, Sheriff MH et al. Speed of initial atropinisation in significant organophosphorus pesticide poisoning--a systematic comparison of recommended regimens. J Toxicol Clin Toxicol 2004;42(6):865-75.

(6) Wilson IB, Ginsburg S. A powerful reactivator of alkylphosphate-inhibited acetylcholinesterase. Biochem Biophys Acta 1955;18:168-70.

(7) Namba T, Hiraki K. PAM (Pyridine-2-aldoxime methiodide) therapy for alkylphosphate poisoning. J Am Med Ass 1958;166 :1834-9.

(8) Erdmann WD, Clarmann M. Ein neuer Esterase-Reaktivator für die Behandlung von Vergiftungen mit Alkylphosphaten. Deutsche medizinische Wochenschrift 1963;45:2201-7.

(9) Sundwall A. Minimum concentrations of N-methylpyridinium-2-aldoxime methane sulphonate (P2S) which reverse neuromuscular block. Biochemical Pharmacology 1961;8:413-7.

(10) Worek F, Thiermann H, Szinicz L, Eyer P. Kinetic analysis of interactions between human acetylcholinesterase, structurally different organophosphorus compounds and oximes. Biochem Pharmacol 2004;68(11):2237-48.

(11) Thiermann H, Szinicz L, Eyer F, Worek F, Eyer P, Felgenhauer N et al. Modern strategies in therapy of organophosphate poisoning. Toxicol Lett 1999;107:233-9.

(12) Worek F, Reiter G, Eyer P, Szinicz L. Reactivation kinetics of acetylcholinesterase from different species inhibited by highly toxic organophosphates. Arch Toxicol 2002;76:523-9.

(13) Eyer P. Optimal oxime dosage regimen, a pharmacokinetic approach. In: Szinicz L, Eyer P, Klimmek R, editors. Role of Oximes in the Treatment of Anticholinesterase Agent Poisoning.Heidelberg, Berlin, Oxford: Spektrum Akademischer Verlag; 1996. p. 33-51.

(14) Eyer P. Use of obidoxime in OP poisoning. Hum Exp Toxicol 1996;15:-78.

(15) Eyer P, Szinicz L, Thiermann H, Worek F, Zilker T. Testing of antidotes for organophosphorus compounds: experimental procedures and clinical reality. Toxicology 2007;233(1-3):108-19.

(16) Thiermann H, Mast U, Klimmek R, Eyer P, Hibler A, Pfab R et al. Cholinesterase status, pharmacokinetics and laboratory findings during obidoxime therapy in organophosphate poisoned patients. Hum Exp Toxicol 1997;16:473-80.

(17) Worek F, Baecker M, Thiermann H, Szinicz L, Mast U, Klimmek R et al. Reappraisal of indications and limitations of oxime therapy in organophosphate poisoning. Hum Exp Toxicol 1997;16:466-72.

(18) Worek F, Szinicz L, Eyer P, Thiermann H. Evaluation of oxime efficacy in nerve agent poisoning: development of a kinetic-based dynamic model. Toxicol Appl Pharmacol 2005.

(19) Worek F, Eyer P, Szinicz L, Thiermann H. Simulation of cholinesterase status at different scenarios of nerve agent exposure. Toxicology 2007;233(1-3):155-65.

(20) Thiermann H, Szinicz L, Eyer P, Felgenhauer N, Zilker T, Worek F. Lessons to be learnt from organophosphorus pesticide poisoning for the treatment of nerve agent poisoning. Toxicology 2007;233(1-3):145-54.

(21) Pawar KS, Bhoite RR, Pillay CP, Chavan SC, Malshikare DS, Garad SG. Continuous pralidoxime infusion versus repeated bolus injection to treat organophosphorus pesticide poisoning: a randomised controlled trial. Lancet 2006;368(9553):2136-41.

(22) 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.

(23) Thiermann H, Szinicz L, Eyer P, Zilker T, Worek F. Correlation between red blood cell acetylcholinesterase activity and neuromuscular transmission in organophosphate poisoning. Chem Biol Interact 2005;157-158:345-7:345-7.

(24) Eddleston M, Eyer P, Worek F, Mohamed F, Senarathna L, von ML et al. Differences between organophosphorus insecticides in human self-poisoning: a prospective cohort study. Lancet 2005;366(9495):1452-9.

(25) Pfeiffer B. Vergiftungen durch Organophosphate I. MTA Dialog 2005;6:583-5.

(26) Pfeiffer B. Analyzing cholinesterase in organophosphate poisoning. Tecan J 2006;2:22-3.

(27) Eberly JP, Eyer P, Pfeiffer B, Szincz L, Worek F. Modification of the Test-mate ChE cholinesterase test system for use over an extended temperature range. Medical Defense Bioscience Review 2004;1-11.

(28) Eyer F, Meischner V, Kiderlen D, Thiermann H, Worek F, Haberkorn M et al. Human parathion poisoning. Toxicol Rev 2003;22(3):143-63.

(29) Eyer F, Eyer P. Enzyme-based assay for quantification of paraoxon in blood of parathion poisoned patients. Hum Exp Toxicol 1998;17:645-51.

(30) Wolthuis O, Vanwersch RA, van der Wiel HJ. The efficacy of some bis-pyridinium oximes as antidotes to soman in isolated muscles of several species including man. Eur J Pharmacol 1981 March 26;70:355-69.

(31) Smith AP, van der Weil HJ, Wolthuis OL. Analysis of oxime-induced neuromuscular recovery in guinea-pig, rat and man following soman poisoning in vitro. Eur J Pharmacol 1981;70:371-9.

(32) Van der Meer C, Wolthuis OL. The effect of oximes on isolated organs intoxicated with organophosphorus anticholinesterases. Biochem Pharmacol 1965;14:1299-312.

(33) van Helden HP, van der Wiel HJ, Wolthuis OL. Retention of soman in rats, guinea-pigs and marmosets: species-dependent effects of the soman simulator, pinacolyl dimethylphosphinate (PDP). J Pharm Pharmacol 1988;40:35-41.

(34) van Helden HP, van der Wiel HJ, de Lange J, Busker RW, Melchers BP, Wolthuis OL. Therapeutic efficacy of HI-6 in soman-poisoned marmoset monkeys. Toxicol Appl Pharmacol 1992;115:50-6.

(35) Alberts P. A new H-oxime restores rat diaphragm contractility after esterase inhibition in vitro. Eur J Pharmacol 1990;184:191-4.

(36) Thiermann H, Eyer P, Worek F, Szinicz L. Effects of oximes on muscle force and acetylcholinesterase activity in isolated mouse hemidiaphragms exposed to paraoxon. Toxicol 2005;214:190.

(37) Maselli RA, Leung C. Analysis of anticholinesterase-induced neuromuscular transmission failure. Muscle Nerve 1993;16:548-53.

(38) Besser R, Gutmann L, Dillmann U, Weilemann LS, Hopf HC. End-plate dysfunction in acute organophosphate intoxication. Neurology 1989;39:561-7.

(39) Massoulie J, Pezzementi L, Bon S, Krejci E, Vallette FM. Molecular and cellular biology of cholinesterases. Prog Neurobiol 1993;41:31-91.

(40) Brimijoin S. Molecular forms of acetylcholinesterase in brain, nerve and muscle: nature, localization and dynamics. Prog Neurobiol 1983;21:291-322.

(41) Mortensen SR, Brimijoin S, Hooper MJ, Padilla S. Comparison of the in vitro sensivitiy of rat acetylcholinesterase to chlorpyrifos-oxon: What do tissue IC50 values represent? Toxicology and Applied Pharmacology 1998;148:46-9.

(42) Namba T, Nolte CT, Jackrel J, Grob D. Poisoning due to organophosphate insecticides. Acute and chronic manifestations. Am J Med 1971;50:475-92.

(43) Brank M, Zajc-Kreft K, Kreft S, Komel R, Grubic Z. Biogenesis of acetylcholinesterase is impaired, although its mRNA level remains normal, in the glucocorticoid-treated rat skeletal muscle. Eur J Biochem 1998 January 15;251(1-2):374-81.

(44) Grubic Z, Sketelj J, Klinar B, Brzin M. Recovery of acetylcholinesterase in the diaphragm, brain, and plasma of the rat after irreversible inhibition by soman: a study of cytochemical localization and molecular forms of the enzyme in the motor end plate. J Neurochem 1981;37:909-16.

(45) Worek F, Eyer P, Szinicz L. Inhibition, reactivation and aging kinetics of cyclohexylmethylphosphonofluoridate-inhibited human cholinesterases. Arch Toxicol 1998;72(9):580-7.

(46) Spöhrer U, Eyer P. Separation of geometrical syn and anti isomers of obidoxime by ion-pair high-performance liquid chromatography. Journal of Chromatography A 1995;693:55-61.