Review Article

Recent Advances in the Treatment of Nerve Agent Poisoning[1]

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Abstract

The standard treatment of nerve agent poisoning is pretreatment with the carbamate pyridostigmine, and post-exposure therapy with atropine, an oxime (PAM, toxogonin or HI-6) and diazepam. Some of the literature published in 1998 bearing on this treatment procedure, and improvements to it, is reviewed in this document. A detailed study of the pharmacokinetics of pyridostigmine revealed that, for a significant number of individuals, there may be times during the course of pyridostigmine prophylaxis (3 tablets per day), when protection against nerve agents is doubtful. Physostigmine is an alternative to pyridostigmine, and there were 3 reports suggesting that slow-release systems of this carbamate may be feasible. Prophylaxis with enzymes has attracted considerable attention in previous years, but little progress was reported in 1998 on improving the hydrolytic activity (towards nerve agents) of these enzymes. Unmodified human butyrylcholinesterase was shown to be effective against inhaled soman; nerve agents had been given by i.v. injection in previous work.

The selective muscarinic antagonist CEB-1957, and the cholinolytic/anticonvulsant drug procyclidine were studied as possible alternatives to atropine, but without compelling evidence for their superiority over atropine. Similarly, there were no results reported for new oximes that would represent a challenge to the currently approved oximes. Even the promising HLö-7 was studied only for its effect on reactivation of cholinesterases inhibited by nerve agents, whereas reactivation is not a good predictor of efficacy in vivo. On the other hand, three anticonvulsants show promise as improvements over diazepam, and two of these are currently undergoing clinical trials (although for a different neuroprotective indication in each case). These drugs, GK-11 (gacyclidine), HU-211 and TCP, are glutamatergic receptor antagonists. The combination of procyclidine and diazepam also showed promise.

A search for an explanation of the Gulf War syndrome continues, with a recent focus on pyridostigmine as the possible cause, particularly in individuals with genetically-based low levels of the scavenger enzyme butyrylcholinesterase. However, the evidence tends to discount this theory. In another approach, a survey revealed that symptoms of the Gulf War syndrome tended to be more prevalent in service personnel whose period of service in south-west Asia commenced <u>after</u> the conclusion of hostilities, i.e.exposure to pyridostigmine or nerve agents could not have been a factor. A rigorous statistical analysis could not be performed, however, because of the low number of personnel in some of the groups of the comparison.

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Introduction

This paper summarises the findings of papers relevant to treatment of nerve agent poisoning that were published in the calendar year 1998, and is an abbreviated version of the latest in a series of such reviews that have been written and forwarded to selected ADF personnel each year.

The currently accepted optimum treatment of nerve agent poisoning is prophylaxis with the carbamate pyridostigmine, followed by therapy after exposure with atropine, an oxime, and the anticonvulsant diazepam. The paper below is organised under these headings. Additional sections discuss enzymes as an alternative prophylactic approach and possible explanations of the Gulf War syndrome.

One of the oximes favoured by several countries is pyridine aldoxime methyl chloride, abbreviated PAM. It is used as the methylsulphonate salt in the U.K., where it is known as P2S. Another oxime, toxogonin, is sometimes known by its alternative name, obidoxime.

Carbamate prophylaxis

Current ADF policy for pyridostigmine prophylaxis is one 30 mg tablet orally every 8 hours. Marino *et al.* studied the pharmacokinetics and pharmacodynamics (mechanism of action) of this regimen in human volunteers over a 3-week period.[1] Plasma pyridostigmine concentration and red blood cell acetylcholinesterase were measured during this time. The pharmacokinetics of pyridostigmine were found to be both gender and weight dependent. It was also calculated that 30% of individuals may not have red blood cell acetylcholinesterase inhibition >10% at the trough of enzyme inhibition. Inhibition >10% is necessary for protection against nerve agent-induced lethality. Despite this drawback of the current dosage regimen, the authors concluded that it has utility in the possible protection of soldiers against nerve agents.[1]

Physostigmine has advantages over pyridostigmine as a prophylactic drug because of its ability to protect the central nervous system against the effects of the nerve agents. Achieving the right dosage is difficult, however, because of its low bioavailablity, short elimination half-life and narrow therapeutic index. Oral slow-release systems or transdermal systems have been proposed to overcome these drawbacks. Benech *et al.* described a transdermal system using a copolymer of acrylic acid and ethyl vinyl acetate to aid delivery.[2] The system showed promise in experiments on rabbits. Philippens *et al.* reported that another slow-release system (osmotic minipump, in guinea pigs) had the benefit of inducing tolerance to the behavioural and neurophysiological side-effects of physostigmine.[3] No side-effects were observed with this system (in contrast with acutely administered physostigmine), even in the absence of scopolamine which is normally necessary to antagonise the cholinergic side-effects of physostigmine. Both the acute and subchronic prophylactic regimes, when combined with atropine therapy, were able to protect the animals against the lethality due to 3 LD_{50} soman. An osmotic minipump was also used by Meshulam *et al.* in experiments with Beagle dogs, an animal model with cholinergic sensitivity similar to humans.[4] A combination of physostigmine and scopolamine conferred full protection against 2 LD_{50} sarin, in the absence of any therapy, with minimal symptoms of toxicity. Behavioural side-effects of the prophylaxis alone were not reported.

Prophylaxis with enzymes

Human butyrylcholinesterase has been shown earlier to protect mice, rats and monkeys against multiple lethal toxic doses of nerve agents, and to prevent the incapacitation caused by these agents. In these earlier experiments, the nerve agents were administered by bolus intravenous injections. Allon *et al.* investigated a more realistic scenario, namely inhalation of nerve agents.[5] They found that a ratio of human butyrylcholinesterase to soman of 0.11 was sufficient to prevent the manifestation of toxic signs in guinea pigs following exposure to 2.2 times the inhaled LD_{50} dose of soman. A slight increase in this ratio, to 0.15, produced sign-free animals after 2 sequential respiratory exposures with a cumulative dose of 4.5 LD_{50}. The protection achieved was far superior to that from pretreatment with pyridostigmine and post-exposure therapy with atropine, benactyzine and the oxime TMB-4 (which, however, is not the optimal therapy).

Anticholinergic drug

Trovero *et al.* reasoned that an alternative anticholinergic drug to atropine is desirable because of the undesirable ratio of therapeutic efficiency to adverse effects exhibited by atropine.[6] They reported that CEB-1957 reduced the mortality in rats due to 2 LD_{50} sarin at doses 10 times lower than those for atropine. The oxime PAM was administered with the anticholinergic drug in both cases. The study did not report, however, the dose at which CEB-1957 produces side-effects. CEB-1957 was found to have affinities for muscarinic receptor subtypes in the order $M3 \geq M2 > M1$, whereas atropine is non-selective for these subtypes.

Kim *et al.* reported that procyclidine, in conjunction with physostigmine and PAM, was superior to atropine/physostigmine/PAM in protecting mice against lethality due to diisopropylfluorophosphate (DFP).[7] Procyclidine also prevented DFP-induced convulsions. DFP is not a recognised chemical warfare nerve agent, and the study was not militarily realistic in that the antidotes were all injected 10 minutes prior to DFP. Nevertheless, the study is relevant in that procyclidine possesses both central cholinolytic activity and anticonvulsant activity, and this combination of properties in the one molecule may be beneficial in treatment of nerve agent poisoning (See also the discussion on anticonvulsants below).

Oxime

HLö 7 has been the subject of research in recent years as a more effective oxime than those currently in service, and it was further investigated by Worek *et al.*,[8,9] who compared it with HI 6, PAM, TMB-4 and toxogonin in reactivation of human erythrocyte acetylcholinesterase and plasma butyrylcholinesterase inhibited by any of 5 nerve agents. Although HLö 7 performed the best of the 5 oximes overall, the results have little predictive value, as reactivation *in vitro* does not correlate with protection against lethality *in vivo*.

Anticonvulsant

One of the drawbacks of diazepam (valium, a benzodiazepine) is its sedative properties. Tashma *et al.* reasoned that a partial agonist of benzodiazepine receptors (in contrast with diazepam, which is a full agonist) might be an effective anticonvulsant with fewer side-effects. They proposed bretazenil as such a partial agonist, although with little comparative data.[10]

Lallement *et al.* refined an earlier study on the protective effects of GK-11, a non-competitive antagonist of glutamatergic NMDA receptors, which are known to be involved in the maintenance of soman-induced seizures and the subsequent neuropathology.[11] The repeat study was designed to be a more realistic model of a field situation, in that monkeys were pretreated with pyridostigmine, exposed to 8 LD_{50} soman, treated one minute later with the human equivalent of one (but not two) autoinjector of atropine/PAM/diazepam, and then left for 45 minutes before receiving GK-11 i.v. It was found that GK-11 improved survival, prevented soman-induced seizures and motor convulsions, prevented development of neuropathology, and accelerated clinical recovery. The authors proposed that GK-11 represents a promising adjuvant to the currently available emergency therapy for management of organophosphate poisoning in man. GK-11 is presently being evaluated in a human clinical trial for a different neuroprotective indication.

The nonpsychotropic cannabinoid HU-211, another antagonist of glutamatergic neurotransmission in the brain, is also currently being evaluated in clinical trials as a neuroprotectant. Filbert *et al.* found that HU-211 protected rats against soman-induced brain damage, even though it had no effect on soman-induced seizure strength or duration.[12] Previous research had established a correlation between brain damage and the occurrence of seizures.

A third NMDA antagonist, N-[1-(2-thienyl)cyclohexyl]piperidine (TCP), was reported by De Groot *et al.* to be very effective, in conjunction with atropine and pyridostigmine, in treating guinea pigs that experienced soman-induced seizures for at least 30 minutes in the absence of treatment.[13] Seizures, neuropathology and behavioural deficits were prevented by the treatment.

Koplovitz *et al.* proposed that an additional anticholinergic drug be administered in conjunction with diazepam.[14] They administered the anticonvulsant drug combination to pyridostigmine-pretreated guinea pigs (who also received atropine–PAM therapy) 5 or 40 minutes after 2 LD_{50} soman, and found that the treatment was effective in terminating seizures. The anticholinergic drugs found to be effective were scopolamine, biperiden, trihexyphenidyl and procyclidine.

Gulf War Syndrome

Between the invasion of Kuwait by Iraq in August, 1990, and the end of the Gulf War in March, 1991, the U.S.A. had 697 000 military personnel in the Persian Gulf region. Since their return, approximately 30 000 (4.3%) have experienced a variety of complaints including chronic fatigue, muscle and joint pain, gastrointestinal disturbances, ataxia, rash, headache, difficulty concentrating, forgetfulness and irritability. There has been no explanation for these symptoms. Abou-Donia *et al.* suggested that an explanation might lie in synergistic toxicity resulting from simultaneous exposure of the service personnel to pyridostigmine (taken in expectation of a nerve agent attack), the insect repellent DEET (N,N-diethyl-*m*-toluamide) and the insecticide permethrin.[15] Subsequent papers provided evidence inconsistent with this hypothesis. For example, McCain *et al.* observed that the doses necessary for such synergy correspond with an average 70-kg service member simultaneously ingesting 107 pyridostigmine tablets, 23 six-ounce cans of 0.5% permethrin aerosol spray and 6.6 two-

ounce tubes of 33% DEET.[16] Nevertheless, Olson *et al.* devised a study to investigate subtle neurobehavioural effects and neuropathology in rats due to exposure to combinations of low levels of sarin, DEET, chlorpyrifos (another insecticide which was used by some troops), pyridostigmine and botulinum toxoid. Their paper reported the study design only; results have yet to be published.[17]

The involvement of pyridostigmine in the Gulf War syndrome was also investigated by Lallement *et al.*[18] Their study was prompted by an earlier report that stress due to forced swimming allowed penetration of pyridostigmine into the brain of mice. Accordingly, it had been proposed that in troops exposed to emotional stress under the conditions of the Gulf War, the blood-brain barrier may have unexpectedly become permeable to pyridostigmine, thus leading to an increased frequency of CNS symptoms. Lallement *et al.* showed that one cannot generalise based on this one set of experimental conditions.[18] Thus, in their experiments, guinea pigs were exposed to the stress of elevated temperature (such as troops might experience in wearing protective clothing in hot or even moderate climates). Penetration of pyridostigmine into the brain was evaluated directly (using radiolabelled pyridostigmine) and indirectly (inhibition of acetylcholinesterase). No entry of pyridostigmine into the central nervous system could be detected in any circumstance. The authors cautioned against extrapolating results of animal experiments to the human situation. This caution should be kept in mind in evaluating the results of Servatius *et al.*, who described experiments with rats to support their suggestion that individuals with impaired butyrylcholinesterase activity, caused by exposure to stress or as a product of genetics, may have been at greater risk of persistent central nervous system dysfunction after prophylactic pyridostigmine treatment. [19] In these experiments, Wistar-Kyoto rats, but not Sprague-Dawley rats, exhibited a delayed-onset, persistently exaggerated startle response after pyridostigmine, and this response was dose-dependent. Wistar-Kyoto rats have inherently lower activity of butyrylcholinesterase (a scavenger of pyridostigmine) than Sprague-Dawley rats. A link between pyridostigmine and the Gulf War syndrome in individuals with a genetic vulnerability to acetylcholinesterase inhibitors was also proposed by Shen,[20] but the link was based only on speculation, and lacked evidence for the claim. Some inaccurate statements in the paper do not help the author's case.

Kurt employed sophisticated statistical tests in a study of 249 Naval Reserve construction battalion men, and claimed an association between delayed-onset neurotoxicity and exposure to drug-chemical combinations.[21] He drew on the results[15] of Abou-Donia *et al.* and the supposed stress-induced penetration of pyridostigmine into the brain to support his findings. As explained above, there is doubt about the validity of these hypotheses. A contrasting conclusion to that of Kurt was reported by Spencer *et al.*,[22] who conducted a survey of service personnel from north-west U.S.A., and separated the population into 4 groups, based on the timing of their service in south-west Asia :- (a) August – December, 1990 (Desert Shield; pre-combat), (b) January – March, 1991 (Desert Storm; combat period), (c) April – July, 1991 (post-combat), and (d) combinations of these periods. The unexplained illnesses of the Gulf War Syndrome were divided into 3 groups - fatigue, cognitive/psychological, and musculoskeletal symptoms. Analysis of the results was limited by small population numbers in some of the time groups. Nevertheless, there was a trend for all 3 case symptoms to be most prevalent amongst service personnel who served exclusively in the post-combat period. This finding, if replicated in a larger study, indicates that exposure to chemicals is not the cause of the Gulf War syndrome.

Another factor that has been proposed as an explanation of the Gulf War syndrome is exposure to low levels of sarin at Khamisiyah, Iraq in March, 1991. Moore,[23] therefore, reviewed the literature on low-level, asymptomatic exposure to nerve agents in controlled studies of human exposures, reports of accidental exposures, and animal studies. No evidence was found for observable long-term adverse health effects following such exposure.

References

1. Marino MT, Schuster BG, Brueckner RP, Lin E, Kaminskis A, Lasseter, KC. Population pharmacokinetics and pharmacodynamics of pyridostigmine bromide for prophylaxis against nerve agents in humans. *J Clin Pharm* 1998; 38:227-235.
2. Benech H, Vincenti M, Fouchart F, Pruvost A, Vienet R, Istin M, Grognet JM. Development and *in vivo* assessment of a transdermal system for physostigmine. *Methods and Findings in Experimental and Clinical Pharmacology* 1998; 20:489-498.
3. Philippens IHCHM, Busker RW, Wolthuis OL, Olivier B, Bruijnzeel PLB, Melchers BPC. Subchronic physostigmine pretreatment in guinea pigs: Effective against soman and without side effects. *Pharm Biochem Behavior* 1998; 59:1061-1067.
4. Meshulam Y, Cohen G, Chapman S, Alkalai D, Levy A. Prophylaxis against organophosphate poisoning by sustained release of physostigmine and scopolamine. *Bioscience Review - 1998 Proceedings* (Minimizing Chemical Warfare Threat through Development of Advanced Medical Countermeasures) 1998; May 31 – June 4: US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, U.S.A. (CD-ROM).
5. Allon N, Raveh L, Gilat E, Cohen E, Grunwald J, Ashani Y. Prophylaxis against soman inhalation toxicity in guinea pigs by pretreatment alone with human serum butyrylcholinesterase. *Toxicol Sci* 1998; 43:121-128.
6. Trovero F, Brochet D, Breton P, Tambuté A, Bégos A, Bizot J-C. Pharmacological profile of CEB-1957 and atropine toward brain muscarinic receptors and comparative study of their efficacy against sarin poisoning. *Toxicol Appl Pharm* 1998; 150:321-327.
7. Kim Y-B, Shin S, Sok D-E, Kang J-K. Effectiveness of procyclidine in combination with carbamate prophylactics against diisopropylfluorophosphate poisoning. *Envir Toxicol Pharm* 1998; 5:43-49.
8. Worek F, Widmann R, Knopff O, Szinicz L. Reactivating potency of obidoxime, pralidoxime, HI 6 and HLö 7 in human erythrocyte acetylcholinesterase inhibited by highly toxic organophosphorus compounds. *Arch Toxicol* 1998; 72:237-243.
9. Worek F, Eyer P, Szinicz L. Inhibition, reactivation and aging kinetics of cyclohexylmethylphosphonofluoridate-inhibited human cholinesterases. *Arch Toxicol* 1998; 72:580-587.
10. Tashma Z, Raveh L, Liani H, Alcalay D, Givoni S, Kapon J, Cohen G, Alcalay M, Grauer E. Benzodiazepine-receptor partial agonists in the prevention of OP-induced convulsions. *Bioscience Review - 1998 Proceedings* (Minimizing Chemical Warfare Threat through Development of Advanced Medical Countermeasures) 1998; May 31 – June 4. US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, U.S.A. (CD-ROM).
11. Lallement G, Clarencon D, Masqueliez C, Baubichon D, Galonnier M, Burckhart M-F, Peo'ch M, Mestries JC. Nerve agent poisoning in primates: antilethal, anti-epileptic and neuroprotective effects of GK-11. *Arch Toxicol* 1998; 72:84-92.
12. Filbert MG, Forster JS, Smith CD, Ballough GPH. Neuroprotective effects of HU-211 on brain damage resulting from soman-induced seizures. *USAMRICD-TR-98-02* 1998, U.S. Army Medical Research Institute of Chemical Defense.
13. De Groot DMG, Bierman EPB, Bruijnzeel EPB, Carpentier P, Kulig B, Lallement G, Melchers BPC, Philippens I. Beneficial effects of TCP on soman-intoxication in guinea pigs. EEG-seizures, brain damage and learning behaviour. *Bioscience Review - 1998 Proceedings* (Minimizing Chemical Warfare Threat through Development of Advanced Medical Countermeasures) 1998; May 31 – June 4. US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, U.S.A. (CD-ROM).
14. Koplovitz I, Schulz S, Shutz M, Railer R, Macalalag R, Schons M, McDonough J. Combination anticonvulsant treatment for nerve agent seizures. *Bioscience Review - 1998 Proceedings* (Minimizing Chemical Warfare Threat through Development of Advanced Medical Countermeasures) 1998, May 31 – June 4. US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, U.S.A. (CD-ROM).
15. Abou-Donia MB, Wilmarth KR, Jensen KF, Oehme FW, Kurt TL. Neurotoxicity resulting from coexposure to pyridostigmine bromide, DEET, and permethrin: Implications of Gulf War chemical exposures. *J Toxicol Environ Health* 1996; 48:35-56.
16. McCain WC, Lee R, Johnson MS, Whaley JE, Ferguson JW, Beall P, Leach G. Acute oral toxicity study of pyridostigmine bromide, permethrin, and DEET in the laboratory rat. *J Toxicol Environ Health* 1997; 50:113-124.
17. Olson CT, Blank JA, Menton RG. Neuromuscular effects of low level exposures to sarin, pyridostigmine, DEET, and chlorpyrifos. *Drug Chem Toxicol* 1998; 21 (Suppl. 1):149.
18. Lallement G, Foquin A, Baubichon D, Burckhart M-F, Carpentier P, Canini, F. Heat stress, even extreme, does not induce penetration of pyridostigmine into the brain of guinea pigs. *NeuroToxicol* 1998; 19:759-766.
19. Servatius RJ, Ottenweller JE, Beldowicz D, Guo W, Zhu G, Natelson BH. Persistently exaggerated startle responses in rats treated with pyridostigmine bromide. *J Pharm Experiment Therap*1998; 287:1020-1028.
20. Shen Z-X. Pyridostigmine bromide and Gulf War syndrome. *Medical Hypotheses* 1998; 51:235-237.
21. Kurt TL. Epidemiological association in US veterans between Gulf War illness and exposures to anticholinesterases. *Toxicol Letters* 1998; 102-103:523-526.
22. Spencer PS, McCauley LA, Joos SK, Lasarev MR, Schuell T, Bourdette D, Barkhuizen A, Johnston W, Storzbach D, Wynn M, Grewenow R. U.S. Gulf War veterans: Service periods in theater, differential exposures, and persistent unexplained illness. *Toxicol Letters* 1998; 102-103:515-521.
23. Moore DH. Health effects of exposure to low doses of nerve agent – a review of present knowledge. *Drug Chem Toxicol* 1998; 21 (Suppl. 1):123-130.