Chapter 6

PRETREATMENT FOR NERVE AGENT EXPOSURE

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INTRODUCTION

Nerve agents are rapidly acting chemical compounds that can cause respiratory arrest within minutes of absorption. Their speed of action imposes a need for rapid and appropriate reaction by exposed soldiers, their buddies, or medics, who must administer antidotes quickly enough to save lives. A medical defense against nerve agents that depends completely on postexposure antidote treatment, however, has two key limitations:

- In the stress of a chemical environment, even well-trained military personnel will not be uniformly successful in performing such tasks as self- and buddy-administration of nerve agent antidotes.[1]
- Aging, a change over time in the interaction of nerve agents with the target enzyme acetylcholinesterase (AChE), renders oxime therapy (an important component of nerve agent antidotes) much less effective.[2] As explained below, aging poses an especially difficult problem for treating effects from the nerve agent soman.

Because of these limitations of postexposure protection, military physicians have focused on the possibility of protecting soldiers from nerve agents by medical prophylaxis, or pretreatment, designed to limit the toxicity of a subsequent nerve agent exposure. A significant problem with pretreatments, however, has been their own potential for adverse effects. In general, the pharmacological pretreatments that protect humans from the toxic effects of nerve agents are themselves neuroactive compounds. Thus, their principal adverse actions are neurological as well and may impair physical and mental performance. A pretreatment must be administered to an entire force under a nerve agent threat. Any resulting performance decrement, even a comparatively minor one, would make pretreatment use unacceptable in battlefield situations requiring maximum alertness and performance for survival.

In the late 1980s, the United States, following the example of Great Britain, stocked the compound pyridostigmine for its combat units as a wartime contingency pretreatment adjunct for nerve agent exposure.[3] Several other Allies, including most members of the North Atlantic Treaty Organization (NATO), did so as well. At the recommended dose, pyridostigmine is free of performance-limiting side effects. Unfortunately, pyridostigmine by itself is ineffective as a pretreatment against subsequent nerve agent exposure and thus it is not a true pretreatment compound. Pyridostigmine pretreatment does provide greatly improved protection against soman exposure, however, when combined with postexposure antidote therapy. For this reason, pyridostigmine is classified as a pretreatment adjunct.

Research workers have attempted to develop true nerve agent pretreatments whose own neurotoxicity is balanced or diminished by coadministration of a pharmacological antagonist to their undesirable properties (eg, the carbamate compound physostigmine, which is administered in combination with a cholinolytic compound, such as scopolamine). The potential and the problems of this pretreatment approach are considered in this chapter, along with a new pretreatment concept that involves inactivating or binding nerve agents with scavenger macromolecules in the circulation.

AGING OF NERVE AGENT–BOUND ACETYLCHOLINESTERASE

Organophosphate nerve agents inhibit the active site of AChE, a key enzymatic regulator of cholinergic neurotransmission. As noted in Chapter 5, Nerve Agents, agent-bound AChE can be reactivated by a class of antidote compounds, the oximes, which remove the nerve agent molecule from the catalytic site of AChE.

During the attachment of the agent with the enzyme, a portion of the agent—the leaving group—breaks off. During a second, later reaction, one of the nerve agent's alkyl groups leaves: this is the process known as *aging*. The rate at which this dealkylation of the AChE-bound nerve agent molecule proceeds depends on the nature of the nerve agent. Table 6-1 shows the aging half-time of each of the five chemical compounds commonly considered to be nerve agents: tabun (GA), sarin (GB), soman (GD), GF, and VX.

Aging is an irreversible reaction. After dealkylation, an AChE-bound nerve agent molecule can no longer be removed from the enzyme by an oxime. Thus, aging of enzyme-bound nerve agent prevents oxime antidotes from reactivating AChE. This is an extremely difficult problem in the

TABLE 6-1

AGING HALF-TIME OF NERVE AGENTS

Nerve Agent	RBC-ChE Source	Aging Half-Time
GA (Tabun)	Human (in vitro)	>14 h[1]
	Human (in vitro)	13.3 h[2]
GB (Sarin)	Human (in vivo)	5 h[3]
	Human (in vitro)	3 h[1]
GD (Soman)	Marmoset (in vivo)	1.0 min[4]
	Guinea pig (in vivo)	7.5 min[4]
	Rat (in vivo)	8.6 min[4]
	Human (in vitro)	2–6 min[1]
GF	Human (in vitro)	40 h[1]
	Human (in vitro)	7.5 h[5]
VX	Human (in vivo)	48 h[3]

RBC-ChE: erythrocyte cholinesterase
Data sources: (1) Mager PP. *Multidimensional Pharmacochemistry*. San Diego, Calif: Academic Press; 1984: 52–53. (2) Doctor BP, Blick DW, Caranto G, et al. Cholinesterases as scavengers for organophosphorus compounds: Protection of primate performance against soman toxicity. *Chem Biol Interact*. 1993;87:285–293. (3) Sidell FR, Groff WA. The reactivatibility of cholinesterase inhibited by VX and sarin in man. *Toxicol Appl Pharm*. 1974;27:241–252. (4) Talbot BG, Anderson DR, Harris LW, Yarbrough LW, Lennox WJ. A comparison of in vivo and in vitro rates of aging of soman-inhibited erythrocyte acetylcholinesterase in different animal species. *Drug Chem Toxicol.* 1988;11:289–305. (5) Hill DL, Thomas NC. *Reactivation by 2-PAM Cl of Human Red Blood Cell Cholinesterase Poisoned in vitro by Cyclohexylmethylphosphonofluoridate (GF)*. Edgewood Arsenal, Md: Medical Research Laboratory; 1969. Edgewood Arsenal Technical Report 43-13.

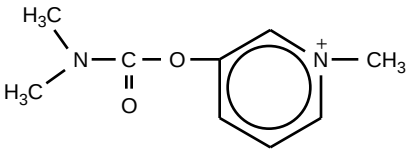
case of poisoning with soman, which ages within 2 minutes.

Aging appears to be a key limiting factor in the efficacy of postexposure oxime therapy for soman poisoning. One method for assessing the relative efficacy of antidotes and other countermeasures is the determination of their protective ratios. The protective ratio (PR) of an antidote is the factor by which it raises the LD_{50} or the LCt_{50} of a toxic nerve agent challenge. Readers will remember that LD_{50} is defined as the dose (D) of liquid or solid nerve agent that is lethal (L) to 50% of the subjects exposed to it; LD_{50} is also described as the median lethal dose. LCt_{50} is the term used to describe the median lethal concentration for an aerosol or vapor agent, expressed as concentration (C) • time (t) of exposure (mg • min). For example, a PR of 1.0 would indicate a completely ineffective antidote, because it means that the LD_{50} or LCt_{50} is the same for subjects who received an antidote and those who did not. A PR of 5, on the other hand, indicates that the LD_{50} or LCt_{50} for subjects who received an antidote is 5-fold higher than that for subjects who did not receive one. A PR of 5 or greater is considered to represent a reasonable level of effectiveness for medical countermeasures against nerve agents. This value was determined through threat analysis of battlefield conditions and consideration of the fact that trained and equipped soldiers will be able to achieve at least partial protection against nerve agent attacks by rapid donning of masks and use of chemical protective clothing.

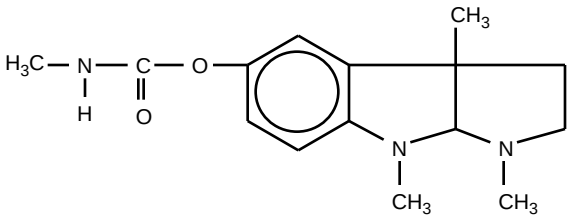
PYRIDOSTIGMINE, A PERIPHERALLY ACTING CARBAMATE COMPOUND

Pyridostigmine is one of a class of neuroactive compounds called carbamates. Its chemical structure and that of a related carbamate, physostigmine, are shown below. Like the nerve agents, carbamates inhibit the enzymatic activity of AChE. As a quaternary amine, pyridostigmine is ionized under normal physiological conditions and penetrates poorly into the central nervous system (CNS). Pyridostigmine has been used for many years in the therapy of neurological disorders, especially myasthenia gravis, a disease of neuromuscular transmission. In patients with myasthenia gravis, inhibition of synaptic AChE is clinically beneficial.

As an inhibitor of AChE, pyridostigmine in large doses mimics the peripheral toxic effects of the or-



Pyridostigmine



Physostigmine

ganophosphate nerve agents. At first it might seem paradoxical that carbamate compounds should help protect against nerve agent poisoning, but two critical characteristics of the carbamate–enzyme bond explain the usefulness of the carbamates.

First, carbamoylation, the interaction between carbamates and the active site of AChE, is freely and spontaneously reversible, unlike the normally irreversible inhibition of AChE by the nerve agents. No oxime reactivators are needed to dissociate, or decarbamoylate, the enzyme from a carbamate compound. Carbamates do not undergo the aging reaction of nerve agents bound to AChE.

Second, carbamoylated AChE is fully protected from attack by nerve agents because the active site of the carbamoylated enzyme is not accessible for binding of nerve agent molecules. Functionally, sufficient excess AChE activity is normally present in synapses so that carbamoylation of 20% to 40% of the enzyme with pyridostigmine does not significantly impair neurotransmission.

When animals are challenged with a lethal dose of nerve agent, AChE activity normally decreases rapidly, becoming too low to measure. In pyridostigmine-pretreated animals with a sufficient quantity of protected, carbamoylated enzyme, spontaneous decarbamoylation of the enzyme regenerates enough AChE activity to sustain vital functions, such as neuromuscular transmission to support respiration. Prompt postexposure administration of atropine is still needed to antagonize acetylcholine (ACh) excess, and an oxime reactivator must also be administered if an excess of nerve agent remains to attack the newly uncovered AChE active sites that were protected by pyridostigmine.

Efficacy

Exposure of humans to soman is virtually unknown in Western countries, with the exception of a single laboratory accident.[4] The decision to provide military forces with pyridostigmine is therefore based on a series of animal efficacy studies[5–7] conducted with several species in a number of countries that found evidence that pyridostigmine pretreatment strongly enhances postexposure antidote therapy for soman poisoning.

Data from one experiment are shown in Table 6-2. In this study[7] with male rhesus monkeys, pretreatment with orally administered pyridostigmine inhibited circulating red blood cell AChE (RBC-AChE) by 20% to 45%. (Inhibition of RBC-AChE by pyridostigmine is a useful index of its inhibition of AChE in peripheral synapses). Monkeys that had no pyridostigmine pretreatment were not well protected from soman by the prompt administration of atropine and 2-pyridine aldoxime methyl chloride (2-PAM Cl). The PR of 1.64 in these monkeys is typical of the most effective known postexposure antidote therapy in animals not given pretreatment before a soman challenge. In contrast to this low level of protection, however, the combination of pyridostigmine pretreatment and prompt postchallenge administration of atropine and 2-PAM Cl resulted in greatly improved protection (PR > 40 when compared with the control group; PR = 24 when compared with the group given atropine and 2-PAM Cl).[7]

Limitation of the number of animals available for soman challenge at extremely high doses made accurate calculation of a PR indeterminate in this experiment. The PR was well in excess of 40, clearly meeting the requirement for effectiveness of 5-fold improved protection. In a later study,[8] four of five rhesus monkeys receiving pyridostigmine pretreatment and postexposure therapy of atropine and 2-PAM Cl survived for 48 hours after being challenged with soman at a level 5-fold higher than its LD_{50}.

Pyridostigmine pretreatment shows its strongest benefit (compared with atropine and oxime therapy alone) in animals challenged with soman and tabun and provides no benefit against challenge by sarin or VX.[9–11] Table 6-3 shows the PRs obtained in animals given atropine and oxime therapy after challenge with the five nerve agents with and without

TABLE 6-2

EFFECT OF THERAPY ON LD_{50} IN MONKEYS EXPOSED TO SOMAN

Group	Mean LD_{50}(µg/kg) [95% CL]	Mean Protective Ratio [95% CL]
Control (no treatment)	15.3 [13.7–17.1]	—
Postexposure atropine + 2-PAM Cl	25.1 [22.0–28.8]	1.64 [1.38–19.5]
Pyridostigmine pretreatment + postexposure atropine + 2-PAM Cl	> 617	> 40*

*Indeterminate because of small number of subjects; PR relative to the atropine plus 2-PAM Cl group > 24 (617 ÷ 25.1)
CL: confidence limit (based on a separate slopes model)
LD_{50}: the dose that is lethal to 50% of the exposed population
PR: factor by which the LD_{50} of a nerve agent challenge is raised (in this experiment, the LD_{50} for group given therapy divided by the LD_{50} for control group)
2-PAM Cl: 2-pyridine aldoxime methyl chloride
Adapted from Kluwe WM. Efficacy of pyridostigmine against soman intoxication in a primate model. In: *Proceedings of the Sixth Medical Chemical Defense Bioscience Review*. Aberdeen Proving Ground, Md: US Army Medical Research Institute of Chemical Defense; 1987: 233.

pyridostigmine pretreatment.[9] As shown, pyridostigmine pretreatment is essential for improved survival against soman and tabun challenge. With sarin or VX, depending on the animal system studied, pyridostigmine causes either no change or a minor decrease in PRs, which still indicate strong efficacy of atropine and oxime therapy for exposure to these agents. The data for GF show no benefit from pyridostigmine pretreatment for mice and a small benefit for guinea pigs. The only published data[8] on protection of primates from GF show a PR of more than 5 with pyridostigmine pretreatment and atropine/oxime therapy, but a control group treated with atropine/oxime alone for comparison was not included. Clinical experts from all countries who have evaluated pyridostigmine have concluded from these data that it is an essential pretreatment adjunct for nerve agent threats under combat conditions, where the identity of threat agents is virtually never known with certainty.

Pyridostigmine was used to protect soldiers from an actual nerve agent threat in the Persian Gulf War. NATO Allies using pyridostigmine followed their national policies on chemical protection. British soldiers, for example, were ordered to take pyridostigmine for over a month while they were positioned near the Iraqi border. U.S. forces followed the doctrine of only using pyridostigmine when a nerve agent threat was assessed to be imminent by the responsible division- or corps-level commander. Thus, soldiers of the U.S. XVIII Airborne Corps took pyridostigmine for several days in January 1991 until it was determined that SCUD missiles fired against them did not have chemical loads. Later, U.S. ground forces attacking into Iraq and Kuwait used pyridostigmine only as long as the corps-level commanders on the ground considered the Iraqi chemical capability a threat.

U.S. and Allied decisions to use pyridostigmine followed established doctrine, taking into account Iraqi capabilities and intentions. Iraq was known to have substantial stocks of sarin and VX, for which pyridostigmine pretreatment is unnecessary, as discussed above. However, Iraq was also known to be keenly interested in acquiring any compounds that might defeat Allied protection, such as soman. The security of Warsaw Pact stocks of soman, for example, was a growing concern in 1990.

In 1990, it was also known that Iraq had begun large-scale production of GF, a laboratory compound that had not earlier been manufactured in weapons quantity. International restrictions on the purchase of chemical precursors of the better-known nerve agents may have led Iraq to acquire cyclohexyl alcohol, which it then was able to use to produce GF. Very limited data on medical protection against GF were not reassuring. Although GF's aging time with AChE was reported to be relatively long (see Table 6-1), unpublished information from Allied countries suggested that postexposure atropine/oxime therapy in rodents exposed to GF did not protect against the effects of GF poisoning. As confirmed by the later studies shown in Table 6-3, atropine/oxime therapy only provided rodents

TABLE 6-3

EFFECT OF THERAPY WITH AND WITHOUT PYRIDOSTIGMINE PRETREATMENT ON PROTECTIVE RATIOS IN ANIMALS EXPOSED TO NERVE AGENTS

		Protective Ratio	
Nerve Agent	**Animal Tested**	**Atropine + Oxime**	**Pyridostigmine + Atropine + Oxime**
GA (Tabun)	Rabbit	2.4	3.9[1]
	Mouse	1.3	1.7 / 2.1*[2]
	Guinea pig	4.4	7.8 / 12.1*[2]
	Rabbit	4.2	> 8.5[3]
GB (Sarin)	Mouse	2.1	2.2 / 2.0*[2]
	Guinea pig	36.4	34.9 / 23.8*[2]
GD (Soman)	Mouse	1.1	2.5[4]
	Rat	1.2	1.4[5]
	Guinea pig	1.5	6.4 / 5.0*[6]
	Guinea pig	2.0	2.7 / 7.1*[7]
	Guinea pig	1.9	4.9[8]
	Guinea pig	1.7	6.8[9]
	Rabbit	1.4	1.5[1]
	Rabbit	2.2	3.1[4]
	Rabbit	1.9	2.8[3]
	Rhesus monkey	1.6	> 40[10]
GF	Mouse	1.4	1.4[11]
	Guinea pig	2.7	3.4[11]
	Rhesus monkey	—	> 5[12]
VX	Mouse	7.8	6.0 / 3.9*[2]
	Rat	2.5	2.1[5]
	Guinea pig	58.8	47.1 / 45.3*[2]

*Two doses of pyridostigmine were used.

Data sources: (1) Joiner RL, Dill GS, Hobson DW, et al. Task 87-35: Evaluating the efficacy of antidote drug combinations against soman or tabun toxicity in the rabbit. Columbus, Oh: Battelle Memorial Institute; 1988. (2) Koplovitz I, Harris LW, Anderson DR, Lennox WJ, Stewart JR. Reduction by pyridostigmine pretreatment of the efficacy of atropine and 2-PAM treatment of sarin and VX poisoning in rodents. *Fundam Appl Toxicol*. 1992;18:102–106. (3) Koplovitz I, Stewart JR. A comparison of the efficacy of HI6 and 2-PAM against soman, tabun, sarin, and VX in the rabbit. *Toxicol Lett*. 1994;70:269–279. (4) Sultan WE, Lennox WJ. *Comparison of the Efficacy of Various Therapeutic Regimens, With and Without Pyridostigmine Prophylaxis, for Soman (GD) Poisoning in Mice and Rabbits*. Aberdeen Proving Ground, Md: US Army Chemical Systems Labororatory; 1983. ARCSL Technical Report 83103. (5) Anderson DR, Harris LW, Woodard CL, Lennox WJ. The effect of pyridostigmine pretreatment on oxime efficacy against intoxication by soman or VX in rats. *Drug Chem Toxicol*. 1992;15:285–294. (6) Jones DE, Carter WH Jr, Carchman RA. Assessing pyridostigmine efficacy by response surface modeling. *Fundam Appl Toxicol*. 1985;5:S242–S251. (7) Lennox WJ, Harris LW, Talbot BG, Anderson DR. Relationship between reversible acetylcholinesterase inhibition and efficacy against soman lethality. *Life Sci*. 1985;37:793–798. (8) Capacio BR, Koplovitz I, Rockwood GA, et al. *Drug Interaction Studies of Pyridostigmine With the 5HT3 Receptor Antagonists Ondansetron and Granisetron in Guinea Pigs*. Aberdeen Proving Ground, Md: US Army Medical Research Institute of Chemical Defense; 1995. USAMRICD Training Report 95-05. AD B204964. (9) Inns RH, Leadbeater L. The efficacy of bispyridinium derivatives in the treatment of organophosphate poisoning in the guinea pig. *J Pharm Pharmacol*. 1983;35:427–433. (10) Kluwe WM. Efficacy of pyridostigmine against soman intoxication in a primate model. In: *Proceedings of the 6th Medical Chemical Defense Bioscience Review*. Aberdeen Proving Ground, Md: US Army Medical Research Institute of Chemical Defense; 1987: 227–234. (11) Stewart JR, Koplovitz I. The effect of pyridostigmine pretreatment on the efficacy of atropine and oxime treatment of cyclohexylmethylphosphonofluoridate (CMPF) poisoning in rodents. Aberdeen Proving Ground, Md: US Army Medical Research Institute of Chemical Defense; 1993. Unpublished manuscript. (12) Koplovitz I, Gresham VC, Dochterman LW, Kaminskis A, Stewart JR. Evaluation of the toxicity, pathology, and treatment of cyclohexylmethlyphosphonofluoridate (CMFF) poisoning in rhesus monkeys. *Arch Toxicol*. 1992;66:622–628.

with PRs in the range of 1.4 to 2.7. The only primate data available showed that rhesus monkeys given pyridostigmine pretreatment and atropine/oxime therapy uniformly survived a 5-LD_{50} challenge with GF.[8] Concern about Iraq's new GF capability, added to its known interest in acquiring soman, made Allied use of pyridostigmine a reasonable course of action.

The fact that pyridostigmine inhibits AChE has raised one theoretical problem with its use: if 20% to 40% of AChE has been inhibited by pyridostigmine, would a subsequent low-level exposure to a nerve agent, which might be well tolerated with no pretreatment, be converted to a toxic dose if it raised the total percentage of AChE inhibition into a toxic range? In practice, it has not been possible to clearly demonstrate such additive toxicity in animal experiments, perhaps because the increase in nerve agent toxicity from initial signs to lethality rises very sharply over a narrow exposure range. A minor additive toxicity effect would therefore be difficult to detect. The signs of mild nerve agent exposure are easily managed with antidote therapy, and the benefit of a pretreatment in life-threatening exposures is so great as to clearly warrant pyridostigmine pretreatment for soldiers whose exact extent of nerve agent exposure is not predictable.

The fact that an ionized, hydrophilic carbamate compound such as pyridostigmine is effective as a pretreatment adjunct against soman suggests that its critical sites of action and, therefore, the critical sites where soman exerts its lethal effects, are outside the blood–brain barrier. As noted in Chapter 5, Nerve Agents, respiratory arrest after lethal nerve agent exposure appears to be a summation of the agent's effects on tracheobronchial secretions and bronchoconstriction with obstruction, impairment of neuromuscular transmission with respiratory muscle insufficiency, and direct depression of central respiratory drive. Electrophysiological monitoring suggests that of these processes, central respiratory drive may be the most susceptible to nerve agent toxicity.[12]

The effectiveness of pyridostigmine pretreatment may not be conclusive evidence against the importance of central mechanisms in respiratory arrest; it appears that there is at least partial permeability of the blood–brain barrier to polar compounds such as pyridostigmine, specifically in the regions of the fourth ventricle and brainstem, where respiratory centers are located. In addition, an increase in blood–brain barrier permeability occurs rapidly after soman administration.[13,14] The key observation remains that animals pretreated with pyridostigmine that receive atropine and oxime therapy promptly after an otherwise lethal soman exposure are able to maintain adequate respiration and survive.

The major deficiency of pyridostigmine pretreatment is also related to its poor penetration into the brain. Animals that survive challenge with a supralethal dose of nerve agent because of pyridostigmine pretreatment frequently show severe histological evidence of brain injury, prolonged convulsions, and long-lasting performance impairments.[15] Although centrally acting carbamate pretreatment compounds, such as physostigmine, offer a degree of protection against nerve agent–induced brain injury, pretreatment with known brain-protecting compounds such as physostigmine, the benzodiazepine anticonvulsants, and benactyzine has not been acceptable because of their known decremental effects on performance. Postexposure anticonvulsant therapy appears to be the most practical, readily available approach to minimizing nerve agent–induced brain injury and promoting rapid recovery of normal function after severe nerve agent exposure (for further discussion, see Chapter 5, Nerve Agents).

Safety

Pyridostigmine has had a good safety record over the years of its administration to patients with myasthenia gravis. Known adverse reactions have been limited to infrequent drug rashes after oral administration and the complete set of signs of peripheral cholinergic excess, which have been seen only when the dosage in patients with myasthenia gravis was increased to AChE inhibition levels well beyond the 20% to 40% range desired for nerve agent pretreatment. The effects of excessive pyridostigmine—miosis, sweating, intestinal hypermotility, and salivation—could clearly degrade soldiers' performance.

When the recommended adult dose of 30 mg of pyridostigmine bromide, one tablet orally every 8 hours, has been followed, no significant decrements have been found in the performance of a variety of military tasks. A review of British studies reported[16] that pyridostigmine caused no changes in memory, manual dexterity, vigilance, day and night driving ability, or in psychological tests for cognitive and psychomotor skills. No significant changes in sensory, motor, or cognitive functioning at ground level, at 800 ft, and at 13,000 ft were noted in 12 subjects in another study[17] after their fourth 30-mg dose of pyridostigmine.

The flight performance of subjects taking pyridostigmine in two studies[18,19] was not affected, no impairment in neuromuscular function was noted in a study[20] in which subjects took pyridostigmine for 8 days, and cardiovascular and pulmonary function were normal at high altitudes in pyridostigmine-treated subjects in another study.[21] However, one study[22] noted a slight decrement in performance in subjects taking pyridostigmine when they tried to perform two tasks at the same time; these subjects also had a slight decrement on a visual probability monitoring task. Two studies[23,24] found an increase in sweating and a decrease in skin blood flow in pyridostigmine-treated subjects subjected to heat/work stress.

Although there has been wide experience with long-term administration of pyridostigmine to patients with myasthenia gravis, until recently there was no comparable body of safety data in healthy young adults. Short-term pyridostigmine administration (one or two 30-mg doses) has been conducted in peacetime in some countries, including the United States, to screen critical personnel, such as aircrew, for unusual or idiosyncratic reactions, such as drug rash. The occurrence of such reactions appears to be well below the 0.1% level, and no military populations are now routinely screened with administration of a test dose of pyridostigmine.

Pyridostigmine for military use by the United States is approved only as a wartime contingency measure. After the Persian Gulf War, there was much discussion about the use of pyridostigmine under an Investigational New Drug (IND) application.[25–32] The Food and Drug Administration (FDA) waived informed consent for its use to make the best medical treatment available in a specific combat situation.[26] The FDA based this waiver on (*a*) data from animal studies conducted in both the United States and other NATO countries that found that pyridostigmine increases survival when used as pretreatment against challenge by certain nerve agents (data on efficacy in humans challenged by nerve agents is not experimentally obtained), and (*b*) a long history of safety when the drug was used for approved indications at doses severalfold higher than the doses administered in the military. Rarely considered in postwar discussions was the ethical issue of nonuse: If pyridostigmine had not been used, and Iraq had used nerve agents causing large numbers of casualties, should the military have been held responsible for withholding this drug?

A limited number of animal studies of toxicological abnormalities and teratogenicity and mutagenicity in animals that were given pyridostigmine have had negative results (Hoffman-LaRoche, proprietary information).[33] In a study[34] in which pyridostigmine was administered to rats, either acutely or chronically, in doses sufficient to cause an average 60% AChE inhibition, ultrastructural alteration of a portion of the presynaptic mitochondria at the neuromuscular junction resulted, as well as alterations of nerve terminal branches, postsynaptic mitochondria, and sarcomeres. These morphological findings, which occurred at twice the AChE inhibition level desired in humans, have not been correlated with any evidence of functional impairment at lower doses, but they emphasize the need to limit enzyme inhibition to the target range of 20% to 40%. Pyridostigmine has been used by pregnant women with myasthenia gravis at higher doses and for much longer periods than it was used during the Persian Gulf War and has not been linked to fetal malformations.[35] Because safety in pregnancy has not been completely established, the Food and Drug Administration considers pyridostigmine a Class C drug (ie, the risk cannot be ruled out).

Several studies have sought information on pyridostigmine use under certain conditions: soldiers in combat who frequently take other medications; wounding and blood loss; and use while undergoing anesthesia. The possible interaction of pyridostigmine with other commonly used battlefield medications was reviewed by Keeler.[36] There appears to be no pharmacological basis for expecting adverse interactions between pyridostigmine and commonly used antibiotics, anesthetics, and analgesic agents. In a study[37] of pyridostigmine-treated swine, for example, the autonomic circulatory responses to hemorrhagic shock and resuscitation appeared normal. One potentially important effect of pyridostigmine deserves consideration by field anesthesiologists and anesthetists using muscle relaxants for anesthesia induction: depending on the duration of muscle-relaxant administration, there may be either up- or down-regulation of postsynaptic ACh receptors.[36] Clinical assessment of the status of neuromuscular transmission using a peripheral nerve stimulator should provide a basis for adjusting the dose of both depolarizing and nondepolarizing muscle relaxants to avoid an undesirable duration of muscle paralysis.

Wartime Use

Pyridostigmine bromide tablets, 30 mg, to be taken every 8 hours, are currently maintained in war

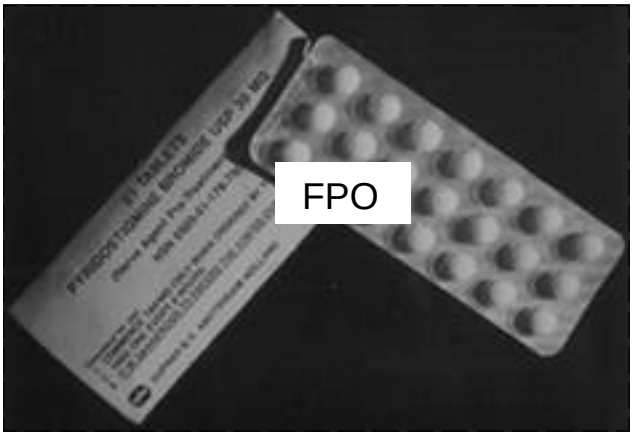


Fig. 6-1. A pyridostigmine blister pack containing 21 30-mg tablets, along with the carrying sleeve. This is the nerve agent pyridostigmine pretreatment set (NAPPS) that was used by designated military personnel during the Persian Gulf War.

stocks of U.S. combat units. The compound is packaged in a 21-tablet blister pack called the nerve agent pyridostigmine pretreatment set (NAPPS, Figure 6-1). One NAPPS packet provides a week of pyridostigmine pretreatment for one soldier.[3]

The decision to begin pretreatment with pyridostigmine is made by commanders at army division level or the equivalent, based on assessment of the nerve agent threat by their chemical, intelligence, and medical staff officers.[3] Because of the lack of data on long-term administration of pyridostigmine to healthy adults, current doctrine calls for a maximum pretreatment period of 21 days, with reassessment at frequent intervals of the need for continued pretreatment. A senior commander's judgment about the severity of a nerve agent threat beyond 21 days determines whether pretreatment should continue.

Pyridostigmine is poorly absorbed when taken orally; its bioavailability is 5% to 10%.[38] Ideally, two doses of pyridostigmine, taken 8 hours apart, should be administered prior to any risk of nerve agent exposure.[3] However, some benefit would be expected even if the first pyridostigmine dose is taken an hour before nerve agent exposure. Because excessive AChE inhibition can impair performance, no more than one 30-mg tablet should be taken every 8 hours. If a dose is forgotten or delayed, administration should simply be resumed on an 8-hour schedule as soon as possible, without making up missed doses.

In Operation Desert Storm in 1991, pyridostigmine was administered under combat conditions for the first time to U.S. and Allied soldiers thought to be at risk for nerve agent exposure. Data on safety and possible adverse responses were collected from the unit medical officers caring for the 41,650 soldiers of the XVIII Airborne Corps who took from 1 to 21 doses of pyridostigmine during January 1991.[39] Most major unit commanders continued the medication for 6 to 7 days, with over 34,000 soldiers taking it for that time. There was nearly total compliance with the regimen by these soldiers, who were fully aware of the nerve agent threat. They were able to perform their missions without any noticeable impairment, similar to findings with peacetime volunteers participating in studies.[16] However, they reported a higher than expected incidence of side effects, as noted in Table 6-4.

Gastrointestinal changes included flatus, loose stools, and abdominal cramps that were noticeable but not disabling. Together with urinary urgency, many soldiers reported a sense of awareness that they were taking a medication. In most soldiers, these changes were noticed within hours of taking the first tablet. In many, the effects subsided after a day or two of administration, and in others they persisted as long as pyridostigmine was administered. Some units adopted a routine of taking pyridostigmine with meals, which was thought to minimize gastrointestinal symptoms.

Soldiers taking pyridostigmine during this period were also experiencing a wide range of other wartime-related stresses, such as repeatedly don-

TABLE 6-4

EFFECTS OF PYRIDOSTIGMINE PRETREATMENT* ON U.S. SOLDIERS IN THE PERSIAN GULF WAR

Effect	Incidence (%) N=41,650
Gastrointestinal symptoms	≥ 50
Urinary urgency and frequency	5–30
Headaches, rhinorrhea, diaphoresis, tingling of extremities	< 5
Need for medical visit	< 1
Discontinuation on medical advice	< 0.1

*Dose was 30 mg pyridostigmine bromide, administered orally every 8 h for 1 to 7 d.

Adapted from Keeler JR, Hurst CG, Dunn MA. Pyridostigmine used as a nerve agent pretreatment under wartime conditions. *JAMA.* 1991;266:694.

ning and removing their chemical protective suits and masks in response to alarms, sleep deprivation, and anticipation of actual combat. Because there was no comparable group of soldiers undergoing identical stresses without taking pyridostigmine, it is not clear to what extent pyridostigmine itself was responsible for the symptoms noted above. The findings are thus a worst-case estimate for effects attributable to pyridostigmine use in wartime.

Among these soldiers, fewer than 1% sought medical attention for symptoms possibly related to pyridostigmine administration (483 clinic visits). Most of these had gastrointestinal or urinary disturbances. Two soldiers had drug rashes; one of them had urticaria and skin edema that responded to diphenhydramine. Three soldiers had exacerbations of bronchospasm that responded to bronchodilator therapy. Because the units of the XVIII Airborne Corps had been deployed to a desert environment for 5 months before pyridostigmine was used, most soldiers with significant reactive airways disease had already developed symptoms and had been evacuated earlier. The consensus among medical personnel more recently arrived was that they saw more pyridostigmine-related bronchospasm in their soldiers, who had not been present in theater as long.

Because of increased exposure to the work-of-breathing requirements of being masked, as well as inhaled dust, smoke, and particles, it was unclear whether pyridostigmine was a major causative factor in those who had bronchospasm at the onset of hostilities. Two soldiers from the XVIII Airborne Corps had significant blood pressure elevations, with diastolic pressures of 110 to 120 mm Hg, that manifested as epistaxis or persistent bleeding after a cut and subsided when pyridostigmine was stopped. Another soldier who took two pyridostigmine tablets together to make up a missed dose experienced mild cholinergic symptoms, self-administered an atropine autoinjector, and recovered fully after several hours. There were no hospitalizations or medical evacuations attributable to pyridostigmine among XVIII Airborne Corps soldiers. In other units, at least two female soldiers, both weighing approximately 45 to 50 kg, noted increased salivation, muscular twitching, severe abdominal cramps, and sweating that prompted medical observation. The symptoms subsided after pyridostigmine was stopped. This experience suggests that cholinergic symptoms may occur in a small number of persons of relatively low body weight.

Later in the Persian Gulf War, more than 200,000 service members took pyridostigmine for 1 to 4 days during the ground attack into Iraq and Kuwait. Their medical experience, as personally reported to us by many unit medical officers, was similar to that reported above. It is now clear that pyridostigmine can be used effectively in large military populations under combat conditions without impairing mission performance. Soldiers must have a clear understanding of the threat and the need for this medication, however. Otherwise, it seems unlikely that they would have the same degree of willingness to accept the gastrointestinal and urinary symptoms noted above or to comply with an 8-hour dosage schedule.

In a group of 213 soldiers in Israel who took pyridostigmine (30 mg every 8 h), 75% reported at least one symptom. Included among these symptoms were excessive sweating (9%), nausea (22.1%), abdominal pain (20.4%), diarrhea (6.1%), and urinary frequency (11.3%). In a smaller group of 21 soldiers, pseudocholinesterase (also called butyrocholinesterase, which is discussed later in this chapter) activity was the same in the 12 who were symptomatic and the 9 who were not symptomatic.[40]

An Israeli soldier who developed cholinergic symptoms after taking pyridostigmine was reported[41] to have a genetic variant of serum butyrocholinesterase. The variant enzyme has low binding affinity for pyridostigmine and other carbamates. The authors of the report suggested that persons who are homozygous for the variant enzyme could therefore show exaggerated responses to anticholinesterase compounds. The soldier had a history of prolonged apnea after receiving succinylcholine premedication for surgery. Persons with similar histories of severe adverse responses to cholinergic medications should be carefully assessed concerning their potential deployability to combat, where they might face either a nerve agent threat or the potential need for resuscitative surgery involving emergency induction of anesthesia[36] using cholinergic medications.

Since the Persian Gulf War, veterans of that conflict have experienced a range of illnesses in themselves, in their spouses, and in children conceived after the conflict. Combinations of symptoms have included fatigue, skin rash, muscle and joint pain, headache, loss of memory, shortness of breath, and gastrointestinal and respiratory symptoms, which could be explained by a variety of conditions, but do not fit readily into a single diagnostic pattern.[42]

The possible interaction of multiple, potentially toxic compounds has generated interest in the context of these problems. With respect to pyridostigmine, one report[43] was published of neurotoxicity in chickens that received pyridostigmine together with large parenteral doses of the insect repellent DEET (diethyltoluamide) and the insecticide permethrin. The relevance of this report is doubtful, because systemic administration of the two interacting compounds to the chickens was at least 10,000-fold in excess of their maximum potential absorption from skin or clothing of soldiers.

Both the National Institutes of Health and the National Institute of Medicine of the National Academy of Sciences established expert panels to evaluate these problems and to suggest an etiology or etiologies. Both panels held public hearings, which included testimony from veterans with the symptoms. The initial reports[44,45] of these panels found no evidence to suggest that pyridostigmine use was related to the problems reported.

Improved Delivery

The currently stocked 30-mg pyridostigmine bromide tablets were purchased for wartime contingency use because of their ready availability. Clearly, the need to maintain an 8-hour schedule of pyridostigmine pretreatment under the conditions of actual or anticipated combat stress is a major practical deficiency in our medical defense against nerve agents.

The United States is considering the development of sustained-release forms of pyridostigmine that would permit maintenance of an adequate level of AChE inhibition with once-daily oral administration. To date, however, no sustained-release preparation has shown sufficient promise to warrant advanced testing. Unfortunately as well, efforts to provide transdermal delivery of pyridostigmine with skin patches have had disappointing results, as would be expected because of the polar nature of the compound.

CENTRALLY ACTING NERVE AGENT PRETREATMENTS

The inability of pyridostigmine to provide protection against nerve agent–induced CNS injury has led to two different pharmacological approaches to protection. The first involves improving postexposure treatment with brain-protecting anticonvulsant compounds, such as benzodiazepines. While these compounds have a clear-cut, intrinsic potential for functional impairment and incapacitation, their administration to casualties who are already incapacitated by nerve agents will not increase the total number of casualties. In fact, clinical observation of nonhuman primates suggests that postexposure therapy with the benzodiazepines diazepam and midazolam actually decreases the time to recovery of consciousness after soman intoxication.[46]

An alternative to postexposure therapy is protection of the CNS with pretreatment compounds that penetrate the blood–brain barrier, such as physostigmine, a tertiary amine that freely enters the CNS. Physostigmine is often used as a model compound for reproducing in laboratory animals the clinical signs of nerve agent intoxication. This nonpolar compound carbamoylates CNS AChE and protects experimental animals from nerve agent challenge more effectively than does pretreatment with pyridostigmine.[47] Another centrally acting carbamate compound, cui-xing-ning, with characteristics that are apparently similar to those of physostigmine, has been evaluated in China.[48]

Neuroactive compounds that penetrate the CNS generally cause some degree of performance impairment in experimental animals, as well as a variable incidence of symptoms, such as nausea and light-headedness, in humans. Even a slight degree of impaired performance of critical battlefield tasks would be life-threatening in itself and therefore would be unacceptable in a pretreatment to be administered to all combatants. A possible solution to this problem is antagonism of the undesirable effects of carbamates, which are generally cholinergic in nature, by simultaneous administration of a cholinolytic pretreatment adjunct, such as atropine, scopolamine, or trihexyphenidyl (Artane, manufactured by Lederle Laboratories, Wayne, NJ). Animals treated with what has been called a behavior-deficit-free combination of physostigmine and a cholinolytic compound, for example, show excellent protection against subsequent nerve agent challenge and rapid clinical recovery of normal function.[49]

In theory, it is possible to offset the side effects of physostigmine and achieve a performance-deficit-free effect by careful titration with a cholinergic blocking drug. The severely limiting factor in developing a physostigmine combination pretreatment for practical use is an unacceptable degree of interindividual variation in the bioavailability of this short half-life compound when administered to humans.[50,51] At present, it would appear necessary

to define, for each recipient, an acceptable dose ratio for physostigmine and a cholinolytic adjunct to avoid performance deficits. The effort required for protecting a total force is clearly beyond our current capability. In the event of a technological breakthrough in individual drug delivery of a well-matched, centrally acting pair of carbamate and adjunct compounds, the possibility of developing centrally acting pretreatments would merit further study.

NEW DIRECTIONS: BIOTECHNOLOGICAL PRETREATMENTS

Until recently, medical defense against nerve agents has focused on preventing or reversing the binding of the agents to AChE, as well as on limiting the effects of the agents on neurotransmission by administration of pharmacological antagonists such as atropine. An intriguing new concept for dealing with nerve agent toxicity involves taking advantage of naturally occurring macromolecules, such as a circulating nerve agent scavenger or a metabolizing enzyme, that would, respectively, bind to or catalyze the hydrolysis of nerve agents. These macromolecules have the potential of providing protection against all effects of nerve agents with minimal side effects, since they would stoichiometrically bind or metabolize a nerve agent before its distribution to the site of toxic effect.

The first evidence that circulating macromolecules have potential for protecting animals from nerve agents came from study of the remarkably broad range of toxic doses of the nerve agents in different animal species. For example, the LD_{50} of soman in mice and rats is about 10-fold higher than the LD_{50} in monkeys or guinea pigs.[52] An enzyme, plasma carboxylesterase, binds to and thus inactivates soman and other nerve agents in the G series (but not VX). The different amounts of this enzyme in the blood of various species can adequately explain their differential sensitivity to the G-series nerve agents.[53]

In addition to carboxylesterase, blood contains two forms of cholinesterase, AChE in the red cells (RBC-AChE) and butyrocholinesterase (BuChE; also called pseudocholinesterase and plasma cholinesterase) in the plasma. Both of these forms of cholinesterase bind and inactivate nerve agents. In preloading experiments in which exogenous AChE from fetal bovine serum or BuChE from equine or human sources was administered to animals (nonhuman primates, mice, or rats) intravenously or intramuscularly, a stoichiometric degree of protection against subsequent nerve agent challenge was provided.[54–57] Investigators supported by the U.S. Army Medical Research Institute of Chemical Defense have recently cloned and expressed the genes for both human AChE and human BuChE.[58,59] Administration of either of these human bioproducts, with a potential plasma half-life of up to 12 days for BuChE, may be able to provide similar protection against nerve agent challenge for humans. The main obstacles to development of these products at the present time appear to be the high cost of production of the quantities involved and the possible need for frequent parenteral administration of a relatively short-lived product.

Another biotechnological protective strategy under active study is the production of monoclonal antibodies with high affinity for nerve agents.[60,61] If a human-derived monoclonal antibody of the immunoglobulin G (IgG) class could be produced, theoretically it would have the advantage of being able to bind and thus protect against a soman challenge in man after administration of about 2 g of antibody protein, similar to the amount of polyclonal antibody routinely administered in 10 mL of standard immune serum globulin. The 6-week plasma half-life of IgG in man would make the use of such a product more acceptable.

Nerve agents, like other reactive small molecules, pass through a high-energy transition state during their reaction with water or with tissue targets such as AChE. By preparing antigens with a geometry that spatially mimics the transition states of these small molecules,[62] researchers have raised antibodies which not only bind to the nerve agent molecules but also catalyze their hydrolysis.[63] These catalytic antibodies have a major advantage over the other bioproducts noted above in that they could continue to inactivate multiple nerve agent molecules. For this reason, the preparation of catalytic antibodies to nerve agents, if successful, may result in the development of a superior, long-term nerve agent pretreatment.

Enzymes found in hepatocytes,[64] neuronal cells,[65] and plasma also hydrolyze nerve agents, albeit comparatively weakly. Study of the requirements for hydrolysis at the enzyme active sites could potentially lead to the design of more efficient hydrolytic enzymes that could be used as catalytic scavengers.[66]

The major reason for interest in biotechnologically derived nerve agent pretreatments lies in their

unique mechanism of action as potential circulating nerve agent scavengers and hydrolytic catalysts. Animals protected against nerve agent challenge with these compounds have shown no evidence of toxicity or performance impairment from the nerve agents.[54–56] Thus, soldiers pretreated with these products might be able to function normally in a chemical environment contaminated with levels of agent below the limits of their circulating protection without requiring the use of masks or protective clothing. The operational advantage that these soldiers would have over opponents encumbered by chemical protective equipment adds considerable appeal to exploring the potential of these newer nerve agent countermeasures.

SUMMARY

The inadequacy of postexposure therapy for nerve agent casualties, particularly those with potentially lethal exposures to soman, has been of great concern. Development of pyridostigmine, a peripherally active carbamate compound, as a nerve agent pretreatment adjunct has substantially improved the ability of the U.S. military to protect its soldiers from the lethal effects of nerve agents. A major deficiency of this pretreatment program—that it does not protect the CNS against nerve agent–induced injury—may be overcome by postexposure administration of anticonvulsants. While centrally acting pretreatments offer more effective protection than does pyridostigmine, their development is limited because of their potential for impairing soldier performance. New research may provide a revolutionary advance in protection against nerve agents with biotechnologically derived pretreatments that bind or inactivate nerve agents in the circulation.

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