**Pharmacologic Prophylaxis against Nerve Agent Poisoning**

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**Abstract**

Nerve agent poisoning is characterized by the rapid progression of toxic signs, including hypotension, tremor, convulsions and profound brain damage. In the political arena of today's world, the threat of nerve agent use against military troops has prompted armies to search for prophylactic protection. The two main strategies for prophylaxis include biological scavengers that can bind or cleave nerve agents before they react with acetylcholinesterase, and antidotes as prophylactic treatment. Pyridostigmine is the current pretreatment for nerve agent poisoning and is in use by most of the armed forces in Western countries. However, since pyridostigmine barely crosses the blood-brain barrier it provides no protection against nerve agent-induced central injury. Pyridostigmine is ineffective when administered without post-exposure treatment adjuncts. Therefore, other directions for prophylactic treatment should be explored. These include combinations of carbamates (reversible AChE inhibitors) and central anticholinergics or NMDA receptor antagonists, benzodiazepines or partial agonists for benzodiazepine receptor, and other central AChE inhibitors approved for Alzheimer's disease. The transdermal route is an alternative way for delivering the prophylactic agent. Administration of prophylaxis can be extended also for civilian use during wartime.

*Prophylaxis is needed due to the rapid onset of nerve agent poisoning and its "aging" process*

Nerve agents are the most toxic of the chemical warfare agents. The classic nerve agents are tabun, sarin, soman, cyclosarin and VX. Their major mode of action is inhibition of synaptic acetylcholinesterase, which prevents hydrolysis of ACh. By this mechanism nerve agents stimulate hyperactivity in cholinergically innervated end organs, inducing an acute life-threatening cholinergic crisis. Muscarinic symptoms include hypersecretion by excretory glands (rhinorrhea, salivation, sweating, abdominal cramps), while nicotinic symptoms include fasciculation and twitching of muscle groups, culminating in fasciculated paralysis. Because ACh is the most widely distributed neurotransmitter in the brain, severe exposure may cause a rapid loss of consciousness, seizures and inhibition of the medullary respiratory center. Nerve agent-induced seizure activity and concomitant motor convulsions can rapidly progress to status epilepticus and profound brain damage, as shown in animal models. The principles of antidotal therapy for nerve agent poisoning have not changed since they were established by the British in the early 1970s, and probably all countries worldwide use the same strategies - namely, pretreatment (e.g., pyridostigmine), along with post-exposure treatment consisting of an anticholinergic drug (mostly atropine sulfate) to counteract the acute cholinergic crisis, an oxime to reactivate any unaged inhibited enzyme, and an anticonvulsant (benzodiazepine) to treat or prevent seizures and resultant neuronal damage [1].

The term “pharmacological prophylaxis” for chemical poisoning refers to the medical countermeasures applied before an exposure in order to protect, at least partially, against the deleterious health effects of the chemical agent. Prophylaxis must be distinguished from pretreatment even though these terms are often used interchangeably in the context of organophosphorus poisoning. Pretreatment is the administration of drugs before poisoning in order to increase the efficacy of treatment given post-poisoning. If, as a result of the pretreatment, further medical treatment is not required after the poisoning, the pretreatment is defined as prophylaxis. From a practical point of view, post-poisoning therapy will always be needed (and administered) in cases of severe poisoning, and therefore pretreatment is probably a better term.

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**Rationale and characteristics**

Why do we need a prophylactic treatment against nerve agents? Exposure to lethal levels of nerve agents will produce toxicities that are precipitate in onset and catastrophic in effect. Therefore, antidotal treatment must be administered immediately by self or ‘buddy-aid’ in order to save lives. Relying completely on post-

\[ AChE = \text{acetylcholinesterase} \]
exposure treatment has two key limitations. First, due to the rapid onset and development of the clinical manifestations and central nervous system damage, nerve agent antidotes might not be administered fast enough. This is especially true in a stressful situation such as a chemical attack. Second, the "aging" process, which occurs due to an irreversible dealkylation in the AChE-nerve agent bond, eliminates the efficacy of post-exposure oxime therapy, especially for soman poisoning (soman-inhibited enzyme ages within minutes, in contrast to other volatile nerve agents that age within hours). Because of these limitations, prophylactic treatment is necessary to reduce the toxicity of nerve agent exposure [2].

An ideal prophylactic treatment is characterized by several elements. First, it should be effective against a wide range of nerve agents and its efficacy should not be dependent on post-exposure treatment. Second, it should have a high safety profile and minimal adverse effects as it may be administered in the military context for a long period before the actual exposure to nerve agents, and any resulting performance decrement or limiting adverse effects would be unacceptable on the battlefield. This is an important requirement, since in order to protect effectively against nerve agents, prophylactic compounds should themselves be neuroactive—i.e., having the potential to impair mental performance. Another important requirement for prophylactic treatment is a convenient treatment regimen with a pharmacokinetic profile that provides sufficient protective blood levels of the drug for a long period. As mentioned earlier, the achievement of such an ideal prophylactic agent is difficult, and post-exposure treatment will be needed in severe cases.

**Basic strategies**

There are two main strategies for prophylactic treatment, based on the mechanism of protection against organophosphates: decreasing concentrations of organophosphates in the blood by inactivating them before they reach AChE (bioscavengers), and protecting AChE against its inhibition (the use of current antidotes).

**Bioscavengers**

Bioscavenger proteins, in general, function either by stoichiometrically binding nerve agents or by catalytically cleaving the organophosphorous substrate into inert products. This approach avoids the side effects associated with current antidotes.

- **Stoichiometric bioscavengers.** These are naturally occurring human proteins that irreversibly bind and sequester organophosphates from the circulation before they reach their physiologic targets (cholinergic synapses). This category includes enzymes such as ChEs and endogenous plasma carboxylesterases, as well as specific antibodies against nerve agents. This approach alters the irreversible nature of the organophosphate-ChE interaction from disadvantageous to advantageous; instead of focusing on the organophosphate as an anti-ChE, one may focus on the ChE as an anti-organophosphate [3]. Using this approach, it was shown that administration of fetal bovine serum AChE or human serum butyrylcholinesterase protected animals against a variety of highly toxic organophosphates without any toxic effects or performance decrements. In order to be an effective scavenger against nerve agent toxicity, enzymes should remain stable in the circulation for long periods, be available in sufficient quantities, and not be immunoreactive. Their main disadvantage is that they are high weight molecules; therefore, the 1:1 stoichiometry implies that a large quantity of enzymes is required to neutralize a small amount of toxicant [4,5].

- **Catalytic bioscavengers.** These bioscavengers (e.g., paraoxonase) neutralize organophosphorus by catalytically cleaving it into biologically inert products. They can be administered in smaller quantities than stoichiometric bioscavengers and produce the same degree of protection. They also have the advantage of not being consumed in the process of nerve agent detoxification and are therefore available to protect against multiple exposures [6].

**The use of current antidotes**

These include anticholinergic agents, oximes, benzodiazepines and peripheral or central reversible AChE inhibitors (Table 1). This article will focus on these antidotes.

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*Pyridostigmine is only a pretreatment and not a "true" prophylaxis*

**Reversible AChE inhibitors**

Pyridostigmine bromide is the current pretreatment adjunct for nerve agent poisoning in most armed forces of Western countries, including Israel. As a quaternary amine, pyridostigmine is ionized under normal physiologic conditions and penetrates poorly into the central nervous system [2]. It is a carbamate compound and thus has the capacity to reversibly bind AChE and render the enzyme unavailable for inhibition by nerve agents. This "shielding fraction"

**Table 1. Antidotes being investigated as prophylaxis for nerve agent poisoning**

<table>
<thead>
<tr>
<th>Group</th>
<th>Examples</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reversible AChE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inhibitors Central</td>
<td>Pyridostigmine, physostigmine, donepezil, rivastigmine</td>
<td>Currently used antidote (Not FDA-approved for AD)</td>
</tr>
<tr>
<td>Peripheral</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central anticholinergic*</td>
<td>Benactyzine, scopoletine, (scopolamine)</td>
<td>*Preferably in combination with reversible AChE inhibitors</td>
</tr>
<tr>
<td></td>
<td>Apotheon, Caramiphen</td>
<td>Not approved in the West</td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>Diazepam, lorazepam</td>
<td>Also NMDA antagonist</td>
</tr>
<tr>
<td></td>
<td>Brexazenil</td>
<td></td>
</tr>
<tr>
<td>Oximes</td>
<td>HI-6</td>
<td>As a transdermal patch (most probably not effective)</td>
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of carbacholylated AChE sustains basic functions through the supply of a de-carbacholylated active enzyme until the enzyme is synthesized de novo. Current pretreatment regimens (30 mg three times a day) allow 20–40% of available enzyme to be bound, a process that does not impair neurotransmission due to the existence of sufficient excess of AChE activity in the body [2].

Survival during the immediate post-exposure period is dependent on post-exposure treatment. The protective ratio, i.e., the factor by which a compound lowers the nerve agent's lethality (calculated as the ratio of the LD₅₀ in pretreated animals to the LD₅₀ in untreated animals) is 2.5–6.8 in small animals challenged with soman after pyridostigmine pretreatment and atropine + oxime treatment, compared with 1.1–1.7 for atropine + oxime treatment alone [7]. In non-human primates exposed to soman, the protective ratio with pyridostigmine is even higher [8]. Pyridostigmine has a good safety profile with only mild peripheral cholinergic signs and symptoms, without interference of mental function performance. In a group of 213 soldiers in Israel who took pyridostigmine during the first Gulf War in 1991, symptoms included nausea (22.1%), abdominal pain (20.4%), diarrheas (6.1%), excessive sweating (9%) and urinary frequency (11.3%) [9]. Although not in line with the safety profile, it was considered to be a possible risk factor for the development of the somewhat vaguely defined illness known as Gulf War Veterans' Illness among U.S. and NATO veterans of that war [10]. A possible explanation is that under stress conditions pyridostigmine was shown to cross the blood-brain barrier and enhance neuronal excitability [11]. Unfortunately, because pyridostigmine does not penetrate the central nervous system (at least under normal physiologic non-stress conditions), it does not provide protection against nerve agent-induced CNS injury. Another disadvantage of pyridostigmine pretreatment is that it did not show any benefit in trials on animals challenged with sarin and VX [12].

In order to achieve CNS protection, other carbamates that cross the blood-brain barrier were investigated. Animal studies utilizing physostigmine, a short-acting tertiary amine carbamate, given as a pretreatment for soman in guinea pigs and other rodents, showed promising results. In comparison studies, physostigmine was found to be more protective than pyridostigmine against the detrimental effects of soman and sarin [13]. Since the main problem with physostigmine is its high toxicity and rapid removal, researchers attempted to supply the drug through a transdermal patch [14]. This will be discussed below. Eptastigmine, a non-approved drug which is a derivat of physostigmine, is more lipophilic and less toxic than physostigmine and was shown to be more protective in soman-poisoned mice (protective ratio 2.1 vs. 1.3 with physostigmine) [15].

Pretreatment combination with the two carbamates, pyridostigmine + physostigmine, did not yield any additional benefit over physostigmine alone, both in the degree of AChE inhibition and improvement of survival rate [16].

**Combinations of anticholinergics and reversible AChE inhibitors**

A shortcoming of neuro-active compounds is that they may partially impair CNS function. A possible solution to the central and peripheral cholinergic adverse effects of carbamate compounds is to antagonize them by the simultaneous administration of cholinolytes. While offsetting the side effects of each, cholinolytes and carbamates act together against organophosphorus intoxication [17]. Harris et al. [18] combined physostigmine pretreatment with several anticholinergics (atropine, benadryl, aperphen, scopolamine, azapropen). Each of the combinations showed high efficacy in preventing organophosphorus-induced lethality and convulsions, with rapid clinical recovery up to normal function. In addition, some of these combinations were effective against sarin and VX intoxication in contrast to physostigmine alone. In another study, pretreatment combination of aperphen and physostigmine had synergetic effects in reducing soman-induced incapacitation in guinea pigs [19]. Although there is much evidence from animal studies to suggest that physostigmine is a useful pretreatment for nerve agents, and at least in theory it is possible to offset its side effects, the drawback is its short biological half-life in humans (30 minutes) and the inter-individual variation in its bioavailability [20]. Therefore, in order to confer long-lasting protection, it must be given frequently and in high oral doses, transdermally or as an ongoing infusion. Indeed, the combination of transdermal physostigmine and scopolamine (Scopoderm TTS) afforded full protection against 2LD₅₀ of soman in pigs and 1.5LD₅₀ in guinea pigs [21], and was shown in a human study to have no significant side effects in behavioral tests (0.3 ng/ml physostigmine with 0.1 ng/ml scopolamine) [22].

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**Other central reversible AChE inhibitors are attractive candidates for nerve agent prophylaxis**

Another prophylactic combination, PANPAL, is composed of pyridostigmine, benadryl (a central anticholinergic) and trihexyphenidyl (another central anticholinergic), and was introduced into the Czech Army [23]. According to the authors, the presence of two anticholinergics suppressed some of the pyridostigmine side effects and allowed an increase in pyridostigmine dose, which produced an increase in its prophylactic activity. PANPAL, given alone, protected against 2.23 and 2.55 LD₅₀ of tabun in rats and mice respectively, and significantly increased the therapeutic efficacy of antidotal post-exposure treatment [23]. It was efficient against other nerve agents as well. No health problems or adverse effects were observed in volunteers following usage of PANPAL [23]. However, as stated by the U.S. Chemical Casualty Care Office and true for all these combinations, there is fear that the administration of a muscarinic blocker to healthy subjects, especially when wearing protective clothes against chemical agents, may lead to elevated

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CNS = central nervous system
heat stress in a hot atmosphere due to inhibition of sweating [24].

Another attractive pretreatment combination for nerve agents is a carbamate (pyridostigmine or physostigmine) and caripran, a central anticholinergic and N-methyl-D-aspartate receptor antagonist. Caripran was approved in the past by the U.S. Food and Drug Administration as an anti-tussive agent and is safe for use [25]. The combination of pyridostigmine and caripran afforded better protection against soman in rats than provided by pyridostigmine and scopolamine, a pure anticholinergic agent with central properties [26]. Although they both prevented lethality and electrographic seizure activity, caripran entirely prevented the known consequent cognitive impairment. A possible explanation for this extra protection attributed to caripran is the involvement of the glutamatergic system, especially through the NMDA receptor, in the clinical pathology of organophosphorus poisoning. McDonough and Shih [27] proposed a three-phase hypothesis for nerve agent-induced seizures. According to this theory, the induction of the seizures is due to cholinergic hyperstimulation (phase 1, the cholinergic phase), followed by other non-cholinergic, mainly glutamatergic, excitatory neurotransmitter systems (phase 2, the combined phase); and if seizure activity is not promptly controlled, these excitatory neurotransmitter systems exert control over the seizure process (phase 3, the non-cholinergic phase). Hence, agents that utilize both anticholinergic and anti-glutamatergic properties are more protective against nerve agents.

**Oximes**

Commonly used AChE reactivators, such as obidoxime (Toxogonin) or pralidoxime (2-PAM), can theoretically be used as prophylactic agents, although this approach has a few pitfalls. To date there is no information regarding the human plasma levels of oximes required to confer protection against nerve agents, at least for obidoxime. Therefore, it is difficult to decide which plasma level attained could suffice for an efficient prophylaxis. When administered orally, oximes are poorly absorbed (2-PAM more than the bis-quaternary obidoxime) and have a broad individual variability, thus necessitating a 5–10 g single dose in order to achieve an apparently therapeutic level [28]. A single oral dose as low as 3 g obidoxime causes undesirable side effects, including a cool sensation in the mouth (menthol-like taste), numbness of the face, headache and generalized weakness, which limit its use in high doses [29].

TMB4 is relatively more toxic than other oximes. Moreover, when given with pyridostigmine, the oxime can reactivate the carbamylated enzyme (“caught” by pyridostigmine) and impair the protective factor provided by pyridostigmine [20]. This problem is especially relevant in high protection-providing doses of the oxime. In order to avoid poor absorption, a transdermal patch of HI-6, an effective oxime against soman, was claimed to be developed by the Czech Army [23]. However, it seems doubtful whether such a patch will provide much help to the soldier. For such a patch to be successful, the oxime needs to penetrate the skin easily and in considerable amounts (the therapeutic dose of oximes is in the 100 mg range). The bis-quaternary HI-6 does not fulfill this prerequisite.

**Benzodiazepines**

The rationale for using benzodiazepines as a prophylaxis is to prevent seizures. Administration of diazepam, clonazepam and nitrazepam 5 hours prior to soman exposure in monkeys prevented the onset of seizure activity, with clonazepam and nitrazepam having the longest effective duration and diazepam the shortest [31]. Most studies compared the efficacy of benzodiazepines when administered post-exposure and not as prophylaxis. Few long-acting benzodiazepines were tested for anti-seizure efficacy, among them a Newspaper (a co-drug of diazepam), diazepam, midazolam and clonazepam [32]. Midazolam was shown to be the most potent and rapidly acting agent in small animals when given either 5 or 40 minutes after seizure onset, and slightly better than diazepam in rhesus monkeys (in combination with pyridostigmine, atropine and pralidoxime) [33]. Although effective, benzodiazepines have adverse effects on task performance in an unexposed person, thereby preventing their use as prophylaxis. The elimination half-life of midazolam and metabolites is short compared with diazepam (1–3 hours vs. 20–99 hours respectively), further limiting its use as a prophylactic agent [34].

Bretazenil, a benzodiazepine partial agonist developed by Roche, conferred prophylactic protection against nerve agents in rats at doses that caused far less incapacitation than diazepam in behavioral studies [35]. This differential activity (effective anticonvulsant with few side effects) is attributed to the ordered physiologic responses of benzodiazepine receptor activation that is dose-dependent (anxiolytic > anticonvulsant > psychomotor disturbance), and the ability of bretazenil to partially activate, although fully occupy, the benzodiazepine receptor in a way that allows only the anxiolytic and anticonvulsant effects. However, bretazenil is not being marketed by the company since it has not proven free of psychomotor effects in humans as initially expected.

**Other central reversible AChE inhibitors**

In the last decade, several relatively long-acting, orally administered, centrally active AChE inhibitors with relatively minor peripheral side effects were approved and marketed for the treatment of Alzheimer’s disease. These AChE inhibitors include rivastigmine and donepezil, which are attractive candidates for nerve agent prophylaxis. Donepezil alone, and in combination with scopolamine, antagonized the decrease in temperature, hypoactivity and induction of diarrhea induced by dichlorophenyl fluorophosphate, an irreversible cholinesterase inhibitor [36]. Another compound, hupéne A, which is presently approved for the treatment of Alzheimer’s disease in China, is a natural alkaloid (isolated from the Chinese club moss, Huperzia serrata) and a reversible inhibitor of AChE at the peripheral and central level. This agent was shown by itself (i.e., without any further injection of atropine or benzodiazepine), both in primates and in small animals, to lower soman-induced lethality (1.3 LD50 in rhesus) and to have central neuroprotective properties [37]. Other beneficial properties, such as a long biological half-life in humans and anti-ChE potency

NMNA = N-methyl-D-aspartate
that is superior to pyridostigmine or physostigmine, also support huperzine A as a pretreatment for nerve agent intoxication (38). Although long-time use of Alzheimer's disease drugs can affect the metabolism of AChE (39), which may influence their long-term efficacy, this consideration is not relevant when related to prophylaxis given to a healthy population and for a short time only (1–2 weeks).

Civilian protection

Currently, prophylactic treatment against organophosphorus is aimed at military personnel, but there is also a need to provide this mode of protection to the general civilian population. In that case, other parameters should be taken into consideration, including the magnitude of adverse effects of the drug in more susceptible subpopulations (e.g., pyridostigmine in patients with obstructive lung disease or conduction disturbances of the heart, and central anticholinergics in extreme ages), interactions with other commonly used medications, and different dose groups according to age. It was recently claimed that age, gender, ethnic origin and body mass index status affect AChE levels, and these parameters may affect dosing policy (40). The potential benefit and modes of application should be assessed against the potential risk, and therefore safety parameters are the leading factor in the risk assessment process.

Summary

Prophylaxis for nerve agent poisoning has to be effective (providing protection against a variety of nerve agents), safe and have a convenient treatment regimen. Pyridostigmine is efficient as a pretreatment drug, i.e., only when followed by post-exposure antidotal treatment, but there is still a need for new prophylactic medications. Several options include combinations of anti-ChE and anticholinergics in various routes of administration, drugs with NMDA receptor antagonist properties, anticonvulsants, and centrally active ChE inhibitors licensed for Alzheimer's disease. Further research is required to improve our pretreatment capabilities.

References

Capsule

Streptococcus prophages and infectivity

Streptococcal diseases have many disguises, ranging from minor sore throats to life-threatening toxic shock. The epidemiology of streptococcal diseases has long been problematic, manifesting as suddenly emerging and disappearing epidemics of disparate syndromes with no apparent therapeutic correlate. In a population-wide genomic study of 11 years with data from 255 isolates from Ontario, Canada, Beres et al. (Proc Natl Acad Sci USA 2004;101:11833) implicated the source of waves of invasive disease to the acquisition or loss of prophages, which rapidly generated unique combinations of virulence genes and their characteristic diseases: toxic shock, bacteremia, or necrotizing fasciitis. However, another 7 year Canadian study of 306 cases of invasive group A streptococcal infections revealed a population-based shift from soft tissue infections to pneumonia, especially in women. Hollm-Delgado and associates (Emerg Infect Dis 2005; 11:77) suggest that underlying conditions in the victims may be causing this shift. They found that the risk of soft-tissue streptococcal infections increased after Varicella infections or drug injection, but ultimately could not explain the increase in pneumonia. However a statistical link could not be made between any particular serotype and specific clinical symptoms. It is possible that a prophage may be at work behind the scenes.

E. Israeli

Capsule

Ear origins

All living mammals have a distinctive ear containing three bones (hammer, anvil, and stirrup) and a single jaw bone. These structures evolved from four or more bones that made up the jaw of their reptilian ancestor in the Mesozoic age. It has been thought that this evolution occurred in a basal mammal, prior to the split of monotremes (the few extant mammals that lay eggs) from marsupials and placentals. Rich et al. show that the ear of the earliest known monotreme, from the Early Cretaceous, has only one bone. Thus, the complex ears of mammals arose separately and converged in different mammalian lineages.

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