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REVIEW PAPER

Prophylaxis Against Nerve Agent Intoxications

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ABSTRACT

The acute toxicity of organophosphates is usually attributed to their irreversible inhibition of an enzyme acetylcholinesterase that hydrolyses the neurotransmitter acetylcholine. The resultant increase in concentration of acetylcholine at the cholinergic synapses of the peripheral and central nervous system, and neuromuscular junction is manifested by over-stimulation of the cholinergic neurotransmission. Current antidotal regimens for organophosphate poisoning consisting of a post-exposure therapy with anticholinergics such as atropine, acetylcholinesterase reactivators (oximes), benzodiazepines have some limitations. Therefore, effective prophylaxis before intoxication is of a special interest. Four fundamental prophylactic methods are: (i) protection of acetylcholinesterase against irreversible inhibition by organophosphates using different reversible inhibitors, (ii) protection against neurotoxic effect of organophosphates using benzodiazepines, memantine, NMDA receptor blockers, (iii) administration of cholinesterase preparations of different sources (sometimes commercially available at present) acting as bioscavengers, and (iv) gene therapy being a new treatment modality under intensive research using enzymes hydrolysing/splitting organophosphates with the aim to eliminate toxic agent before it is transported to the target organs.

Keywords: Cholinesterases, inhibitors, organophosphates, poisoning, prophylaxis, pretreatment, acetylcholinesterase, nerve agents, bioscavengers, gene therapy

1. INTRODUCTION

Organophosphates (OPs) are widely used as pesticides and have thus become environmental contaminants. In addition, some OPs like sarin, soman, VX and tabun are important chemical warfare agents[1]. Their production and testing have been forbidden by the international agreements[2]. Nerve agents are rapidly acting chemicals that can cause respiratory arrest within minutes after their 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 their lives.

The acute toxicity of OPs in mammals is primarily due to their irreversible inhibition of acetylcholinesterase (AChE; EC 3.1.1.7) in the nervous system, which leads to increased synaptic acetylcholine levels. However, the toxic effect of some OPs is not limited to cholinesterase inhibition only. Following cholinergic crisis, changes in noncholinergic parameters such as specific damage of cell membranes are observed[3,4]. In the treatment of OPs poisoning, the combination of anticholinergics like atropine and some AChE reactivators called oximes (obidoxime, pralidoxime, HI-6, etc.) has been used[5-8]. For the treatment of convulsions, benzodiazepines (diazepam,

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etc.) are administered[8]. However, the efficiency of oximes is not satisfactory in the case of soman poisoning, due to the rapid dealkylation (ageing) of AChE. The resulting methylphosphonyl AChE is resistant to the nucleophilic attack of oximes[9,10]. Ageing renders oxime therapy much less effective and poses a specially difficult problem for the treatment of soman poisoning. It was found that pretreatment with certain reversible inhibitors of cholinesterase such as carbamates in conjunction with atropine and oximes gave appreciable protection against poisonings by many OPs, including soman[11,12]. A number of possibilities exist to protect an organism against organophosphate intoxication which are summarised in this paper.

2. PROTECTION POSSIBILITIES

Medical protection against nerve agents that depends completely on post-exposure antidote treatment has some limitations. Because of these limitations of post-exposure protection, military physicians have focused on the possibility of protecting soldiers from nerve agents by medical prophylaxis designed to limit the toxicity of a subsequent nerve agent exposure. The administration of prophylactic antidote is not so simple and has some difficulties[13]. The pharmacological pretreatments that protect human beings from the toxic effects of nerve agents are themselves neuroactive compounds. A pretreatment must be administered to the entire force under a nerve agent threat. Any adverse interference of soldier is unacceptable in battlefield situations requiring maximum alertness and performance for survival.

Nowadays, four fundamental methods for protection of the organisms against toxic OPs exist. These are:

- Protection of acetylcholinesterase (AChE) against irreversible inhibition by OP using suitable reversible inhibitor
- Protection of neurons against OP induced neurotoxicity using suitable drugs
- Displacement of OP using suitable bioscavenger
- Gene therapy to prevent OP intoxication by increasing the specific hydrolytic activity of PON1 paraoxonase/arylesterase enzymes in liver and blood. These enzymes provide a natural barrier against the entry of these agents into the central and peripheral nervous systems, ie, to the target sites of OPs toxic action.

2.1 Reversible Inhibitors of AChE

The prophylaxis against nerve agents is based on the protection of AChE against irreversible inhibition by OPs. Partial inhibition of AChE by some reversible inhibitors protects the enzyme against irreversible inhibition by OPs and, therefore, against the lethal effects of organophosphorous nerve agents.

The possibility to protect AChE against irreversible OP by the pretreatment of reversible inhibitor has been known for many years[14,15]. Several reversible inhibitors were tested, for example mono-and bis-pyridinium salts[16], ketamine[17,18], tacrine (9-amino-1,2,3,4-terahydroacridine)[19,20], huperzine A[21,22] and others. However, the best results were obtained using carbamates, viz., physostigmine and pyridostigmine[23-27]. Physostigmine, a natural alkaloid from the West African shrub *Physostigma venenosum* is prophylactically active. Physostigmine penetrates through the blood-brain barrier and therefore is more efective than pyridostigmine in protecting against the detrimental effects of soman[28]. However, physostigmine is more toxic than pyridostigmine and the difference between its therapeutic and toxic dose is very narrow. In addition, its inhibition effect on AChE is short lived. Since pyridostigmine does not penetrate into the brain, it does not afford protection against centrally initiated seizures and subsequent neuropathology induced by an OP agent such as soman. Nevertheless, pyridostigmine was the first reversible inhibitor of AChE which was used on human being as prophylactic antidote against nerve agents.

Recommended dose of pyridostigmine (pyridostigmine bromide[29,30]) as prophylactic antidote is 30 mg every 8 h. At this recommended dose, pyridostigmine did not show the side effects in the animal efficacy studies conducted in several species in a number of countries. Also, the evidence of pyridostigmine pretreatment strongly enhancing post-exposure antidote therapy for soman poisoning[31,32]

was found. This approach shows the strongest benefit of pyridostigmine pretreatment in comparison with atropine and oxime therapy alone in animals challenged with nerve agents[33-37].

As an inhibitor of AChE, pyridostigmine in large doses mimics the peripheral toxic effects of nerve agents and therefore it might seem paradoxical that carbamate compounds help in protection against nerve agent poisoning. However, two critical characteristics of the carbamate-enzyme bond explain the usefulness of the carbamates: First, carbamoylation, the interaction between the carbamates and the active site of AChE, is reversible, unlike the normally irreversible inhibition of AChE by the nerve agents. No reactivators are needed to dissociate or decarbamoylate the enzyme from a carbamate compound. Carbamates do not undergo the ageing reaction like nerve agents. Second, carbamoylated AChE is resistant to inhibition 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 per cent to 40 per cent of the enzyme with pyridostigmine does not significantly impair cholinergic neurotransmission. When animals are challenged with a lethal dose of nerve agent, AChE activity normally decreases rapidly, becoming too low to be measured. In pyridostigmine pretreated animals with a sufficient quantity of carbamoylated enzyme, spontaneous decarbamoylation of the enzyme regenerates enough AChE activity to sustain vital functions such as neuromuscular transmission to support respiration. Pyridostigmine pretreatment provides improved protection against OP exposure. However, therapeutic effect is better when combined with post-exposure antidote therapy. Prompt post-exposure administration of atropine or other suitable anticholinergic is still needed to antagonise acetylcholine excess, and an oxime reactivator has also to be administered if an excess of nerve agent remains to attack the newly uncovered AChE active sites that were protected by pyridostigmine[34,38-40].

It appears from these results that with a higher dose of pyridostigmine, a higher prophylactic efficacy against nerve agents was observed, but some adverse effects were also expressed. This problem was solved by adding the anticholinergic drugs. This prophylactic combination of pyridostigmine with trihexyphenidyle and benactyzine called PANPAL was developed and introduced into the Czech Armed Forces. This antidote has better prophylactic efficacy in comparison with pyridostigmine alone[41-43]. Nowadays, binary antidotes against OP poisoning have been developed, because the combination of pyridostigmine with a proper anticholinergic agent are more effective and their undesirable effects are suppressed.

It is very important because chronic pyridostigmine administration is able due to these adverse reactions and it is impossible to remove their concern in health problem of Gulf war veterans[44]. It is known that Gulf war veterans began to complain of symptoms such as aching joints, chronic fatigue, memory and concentration loss, headache, depression, anxiety, gastrointestinal disturbances, problems in breathing, frequent coughing, chest and heart pain, hives and chemical sensitivities, eye and vision problems[45]. This collection of symptoms acquired its name as Gulf war illness (GWI) or Gulf war syndrome (GWS).

The toxicity of pyridostigmine is the result of several different characteristics of the compound. Inhibition of cholinesterase causes acetylcholine to have a prolonged effect, leading to overactivation of the parasympathetic pathway. This type of overactivation results in respiratory and muscular problems, as well as salivation, lacrimation, urination, diaphoresis, gastroenteritic cramping, and emesis. A pyridostigmine dose (above 2 mg/kg) was found to be toxic in an experiment carried out on beagle dogs, whose symptoms were gastrointestinal problems, emesis, diarrhea, reddening of the stools, and death caused by intestinal intusseception[46]. These symptoms, though extreme, can be seen in a more mild form in Gulf war veterans, who experience diarrhoea, nausea, and stomach pain. High chronic toxicity of pyridostigmine is one of the reasons why new and safer prophylactic antidotes are sought[47].

2.2 Neuroprotective Substances

Soman inhibited AChE is very difficult to reactivate especially following poisoning with multiple lethal doses of soman. In addition, the soman-AChE complex

ages very rapidly, making it resistant to reactivation and does not undergo spontaneous reactivation. Also, convulsive activity in soman intoxication creates a problem that has been linked to irreversible brain damage. Therefore, prophylactic efficacies of anticonvulsants were studied. The benzodiazepines (diazepam, midazolam, alprazolam, triazolam, clonazepam) were studied, but isolated prophylactic administrations have not produced very good effects[4,48,49]. Some experiments have shown that memantine (1-amino-3,5-dimethyl adamantine) prophylaxis potentiates the therapeutic activity of standard antidotes used in OP intoxications[50-54]. Memantine, a drug is used for the therapy of parkinson's disease, spasticity and other brain disorders, and significantly protected hippocampal and cortical neurons in culture against glutamate and N-methyl-D-aspartate (NMDA) excitotoxicity[54]. In rats a single dose of memantine (18 mg/kg) administered 1 h prior to a subcutaneous injection of a 0.9 LD_{50} dose of soman reduced the severity of convulsions and increased survival. Survival, however, was accompanied by neuronal loss in the frontal cortex, piriform cortex, and hippocampus[53,54].

Other NMDA receptor ion-channel blocker, which was tested as prophylactic antidote against OP poisoning, is dizocilpine (MK-801). Its prophylactic effect is not quite clear. Dizocilpine is a neuroprotective agent like memantine, which is a reversible AChE inhibitor. The (-) form of dizocilpine, pharmacologically less active enantiomer, was the most potent of the two isomers as an AChE inhibitor (K_i for electric eel and rat brain AChE being 6.2 μm and 17.9 μm, respectively, compared with 200 μm and 450 μm, respectively of the (+) form). Both enantiomers premixed with AChE preparations, dose dependently protected the enzyme from inactivation by diisopropylfluorophosphate (DFP)[55].

2.3 Bioscavengers

Another possibility to protect the organisms from the OP toxic effects is the use of scavengers that are able to bind organophosporus compounds. Such suitable bioscavengers are enzymes that are irreversibly inhibited by OPs or are able to hydrolyse OPs. These diminish the level of OP absorbed in the blood. Thus, the concentration of OP attainable at the toxic target sites (peripheral and central nervous system) is significantly decreased. Among all the tested enzymes that were promising candidates as scavengers of highly toxic nerve agents, significant advances were achieved using cholinesterases[56]. Exogenous administration of plasma-derived cholinesterases of both rodent and non-human primate models have been successfully used as a safe and efficacious prophylactic treatment to prevent poisoning by OP compounds[57]. A theoretical model for the protection against organophosporus poisoning has been developed and verified on seven OPs[58]. AChE or BuChE has been considered as acceptable bioscavengers[59-61]. Moreover, pretreatment with butyrylcholinesterase also showed protective effects on AChE inhibition in the brain parts following low-level sarin inhalation exposure[62].

It has been demonstrated that cholinesterases are an effective mode of pretreatment to prevent OP toxicity in different animals. The efficacy of cholinesterases as a bioscavenger of OP can be enhanced by combining enzyme pretreatment with oxime reactivation, since the scavenging capacity extends beyond a stochiometric ratio (1:1) of ChE to OP. Human BuChE (HBuChE) has previously been shown to protect mice, rats, and monkeys against multiple lethal toxic doses of organophosphorus anticholinesterases[63].

Enzyme therapy for the prevention and treatment of poisoning depends on the availability of large amounts of cholinesterases. Of the ChEs evaluated so far, human serum butyrylcholinesterase (HBuChE) has the maximum advantage as a potential candidate for human use. A dose of HBuChE i.v. (200 mg) is envisioned as a prophylactic treatment in human beings that can be protected from exposure of up to 2 lethal doses (LD_{50}) of soman. A large-scale purification of HBuChE from human plasma has been developed[64]. The long-term stability of enzyme obtained by this way was very good but the amount of BuChE in human plasma is very low and its preparation is complicated and costing. Better results offer modern biotechnology methods. Transgenic plants are being evaluated for their efficiency and cost-effectiveness as a system for the bioproduction

of therapeutically valuable proteins[65]. Production of a recombinant isoform of human acetylcholinesterase in transgenic tomato plants has been reported. An active and stable acetylcholinesterase, which retains the kinetic characteristics of the human enzyme. High levels of specific activity were registered in leaves (up to 25 nmol min^{-1} mg $protein^{-1}$) and fruits (up to 250 nmol min^{-1}mg $protein^{-1}$). Another suitable source of cholinesterases is transgenic animal. Protexia™ is a recombinant version of human butyrylcholinesterase from the milk of transgenic goats, developed by Nexia Biotechnologies, Canada (http://www.nexiabiotech.com).

Enzymes hydrolysing OP are present in the organism at physiological conditions. Generally, these enzymes are able to hydrolyse G agents (sarin, soman, etc.) more rapidly than V agents (VX and derivatives). Some of these enzymes were purified to obtain higher hydrolysing activity and to detoxify nerve agents. The connection between the two types of enzymes (cholinesterases and OP hydrolysing enzymes) will be possible with aim of obtaining a modified enzyme splitting OP and simultaneously reacting with cholinesterases[8,66].

2.4 Gene Therapy

The specific hydrolytic activity of PON1 paraoxonase/arylesterase enzymes in liver and blood provides a natural barrier against the entry of OPs into the central and peripheral nervous systems. Inherited differences in the concentrations of PON1 enzyme may determine the extent of susceptibility to OP injury in human beings. The findings of Cowan, *et al*. indicate that boosting serum levels of PON1 enzymes by a gene delivery vector raises the threshold for OP toxicity by hydrolytic destruction before the chemical can enter the brain[67]. Gene therapy to prevent OP intoxication is a probability for the future. Another possibility for the neutralisation of OP in the body are monoclonal antibodies, that have proper characteristics for use as an immunocytochemical reagent of high specificity[68,69].

3. CONCLUSION

At present prophylaxis against nerve agent toxicity makes the use of the reversible binding of carbamate cholinesterase inhibitors such as pyridostigmine to AChE. While the AChE is bound to pyridostigmine, enzyme is protected against the irreversible attack by the nerve agents. Since only a small amount of acetylcholinesterase is required for normal nerve transmission, toxicity is prevented by the constant release of active enzyme from its binding with pyridostigmine. Better prophylactic efficacy without adverse effects was achieved using its combination with two anticholinergics (PANPAL). These two prophylactic antidotes are being used against nerve agent action for the military purposes in the Czech Armed Forces, while the other prophylactic antidotes like cholinesterase bioscavengers and gene therapy, are still experimented in the laboratories.

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