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Review

Multifunctional drugs as novel antidotes for organophosphates' poisoning

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ABSTRACT

Some organophosphorus compounds (OPs) are nerve agents that continue to concern military personnel and civilians as potential battlefield and terrorist threats. Additionally, OPs are used in agriculture where they are associated with numerous cases of intentional and accidental misuse. These toxicants induce an array of deleterious effects including respiratory distress, convulsions and ultimately death. A mechanism involving a rapid and potent inhibition of peripheral and central cholinesterases leading to a massive buildup of acetylcholine in synaptic clefts was suggested as the underlying trigger of the toxic events. Indeed, therapy comprised of an acetylcholinesterase reactivator (i.e., oxime) and a cholinergic antagonist (e.g., atropine) is the accepted major paradigm for protection. This approach yields a remarkable survival rate but fails to prevent neurological and behavioral deficits. Extensive research revealed a complex picture consisting of an early activation of several neurotransmitter systems, in which the glutamatergic plays a pivotal role., Data accumulated in recent years support the concept that multi-targeting of pathways including glutamatergic and cholinergic circuits is required for an effective treatment. Drugs that demonstrate the ability to interact with several systems (e.g., caramiphen) were found to afford a superior protection against OPs as compared to specific antimuscarinic ligands (e.g., scopolamine). Compounds that potently block muscarinic receptors, interact with the NMDA ion channel and in addition are able to modulate σ_1 sites and/or GABAergic transmission seem to represent the emerging backbone for novel antidotes against OP poisoning. Several multifunctional drugs are already used for complex diseases e.g., cancer and depression.

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1. Introduction

Some organophosphorous compounds (OPs) are extremely toxic materials used as chemical weapons that have created a substantial threat on the battlefield. The current working hypothesis is that these agents are incorporated in the armamentaria of both the armed forces and terrorist groups. Furthermore, OPs are widely used in agriculture as pesticides and are associated with numerous cases of intentional and accidental misuses (more than 2,000,000 cases/year) (Tattersall, 2009). Pesticide and those chemical warfare agents belong to two distinct chemical families, namely, phosphates and phosphonates, respectively. While all these compounds are potent ChE inhibitors, the latter OPs exhibit significantly higher toxicity and a faster onset of toxic signs in humans (Tattersall, 2009). Additionally, seizures are considerably more typical to toxicants such as soman compared to parathion. Unlike nerve agents, many pesticides induce delayed polyneuropathy in man due to their interaction(s) with a class of enzymes

Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; ACh, acetylcholine; BEN, benactyzine; CRM, caramiphen; ChE, cholinesterases; DMLs, designed multi-targeted ligands; DTG, 1,3-di-o-tolylguanidine; EAAs, excitatory amino acids; GABA, γ-amino-utyric acid; Glu, glutamate; NBQX, 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F) quinoxaline; NMDA, N-methyl-D-aspartate; OP, organophosphorous; PHY, physostigmine; SCO, scopolamine; TCP, thienylcyclohexylpiperidine; THP, trihexyphenidyl.

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known as neuropathy target esterases (Johnson and Glynn, 1995; Jokanovic et al., 2011). The complexity described emphasizes the need to approach the goal of treating OP poisoning with a multi-faceted strategy.

In this review, the highly toxic nerve agents e.g., soman and sarin will be discussed as examples of OPs. Their destructive effects have been shown repetitively in recent decades. Sarin, one of the most toxic chemical agents, was employed twice against peaceful city dwellers in Japan, resulting in more than 6000 victims. Previously in the Iran–Iraq war, sulfur mustard and nerve gas were used against Iranian soldiers and Iraqi Kurdish civilians, causing a great number of casualties.

Chemical warfare compounds such as soman and sarin exert their toxic effects through the inhibition of cholinesterases (ChEs) (Taylor, 2005). As a result of this activity, large quantities of acetylcholine (ACh) are accumulated in synaptic clefts (Lallement et al., 1992a,b), leading to a progression of noxious signs, including hypersecretions, convulsions, cognitive dysfunction and ultimately death. Thus, the initial actions of drugs designed to protect against OP poisoning were finding means of (a) preventing these agents from binding to ChEs, (b) reactivating OP-inhibited ChEs, and (c) blocking the binding of ACh to muscarinic receptors. A paradigm consisting of a prophylactic treatment with pyridostigmine, a reversible inhibitor of ChE and therapy with an oxime and atropine sulfate, markedly improve survival rates against soman exposure (Berry and Davies, 1970; Leadbeater et al., 1985). However, this regimen does not prevent the OP-mediated central nervous system (CNS) sequel of seizures, convulsions and behavioral deficiencies (Shih and McDonough, 1997). Indeed, soman- or sarin-evoked seizure episodes that are left unchecked, progress to status epilepticus (SE), a condition that generates a complex problem for medical management, including cardiovascular pathologies (Allon et al., 2005; McDonough et al., 1989; Petras, 1981), brain damage and long-term learning and memory impairments (Brown and Brix, 1998). Hence, to improve existing therapeutic regimens, a post-exposure administration of an adjunctive anticonvulsant along with atropine and an oxime was proposed (Dunn and Sidell, 1989; Lipp, 1972). Consequently, the anticonvulsant diazepam was introduced as a needed add-on therapy to counter nerve gas poisoning (see Shih and McDonough, 1997 and refs cited therein). Nevertheless, this strategy was proven to provide insufficient protection against the ensuing brain injury (Shih, 1990). In order to introduce an improved medical protocol for victims of OP poisoning, better insights into OP's mechanism of action are required. Several groups (Shih and McDonough, 1997; Solberg and Belkin, 1997) reported that the glutamatergic system is intimately involved. Furthermore, glutamate receptors' antagonists in general, and N-methyl-D-aspartate (NMDA) blockers in particular, were proposed as potential antidotes against nerve agents.

The neurotoxic effects of excitatory amino acids (EAAs) such as glutamate (Glu) was well established a decade before their putative involvement in OP toxicity was introduced (Olney et al., 1977). As noted years later (Choi, 1992), excitotoxicity refers to the ability of Glu or related EAAs to mediate the death of central neurons under certain conditions such as hypoxia or oxidative stress. Excitotoxicity has significant cellular specificity and, in most cases, is mediated by Glu receptors. In general, NMDA receptors activation may trigger lethal injury more rapidly than either α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) or kainate receptor activation, perhaps reflecting a greater ability to induce calcium influx and subsequent cellular calcium overload.

Excitatory amino acids were implicated in the development of seizures, convulsions and ultimately neuronal injury induced by the cholinesterase inhibitor soman (Wade et al., 1987). Extracellular Glu levels increased in animals with seizures shortly after administration of a convulsive dose of soman (81 μg/kg). As mentioned earlier, NMDA channel blockers such as MK-801 are powerful antidotes against soman intoxication in animals (Braitman and Sparenborg, 1989). The authors stated that "this is the first demonstration of the involvement of the excitatory amino acid neurotransmitter system in seizures and convulsions induced by a cholinesterase inhibitor" (Braitman and Sparenborg, 1989). In contrast, a later study (Shih and McDonough, 1997) measured concurrent changes in EEG and neurotransmitter levels in the CNS of soman-intoxicated animals (1.6× LD_{50}) and recorded no changes in glutamine and Glu. A suppression of aspartate content in the cerebral cortex was detected 20 min after the initiation of seizures, concomitant with an increase in the level of the inhibitory transmitter γ-amino-butyric acid (GABA). Electrophysiological experiments on neurons of the CA1 subfield of rat hippocampal slices examined the effects of sarin on field stimulation-evoked postsynaptic currents mediated by activation of $GABA_A$ receptors (Chebabo et al., 1999). The study revealed an inhibitory effect of sarin at low concentrations (0.3–1.0 nM) on GABAergic postsynaptic currents which was unrelated to ChE inhibition. Notably, the authors conclude that sarin causes a selective reduction in the action potential-dependent release of GABA in the hippocampus. The decrease in the amount of the inhibitory transmitter can account for the occurrence of seizures in intoxicated subjects (Chebabo et al., 1999). In fact, some reports provide evidence that OPs toxic activity is associated with a direct interaction between nerve agents and central GABAergic and/or glutamatergic neurons. It is important to note that McDonough and Shih published an article describing the significance of EAAs in the mechanism of ChE inhibitors' toxicity (McDonough and Shih, 1997). This report deduced that neuronal excitation of the seizure per se perturbs other neurotransmitter systems and that the increase in EAAs' levels reinforces the seizure. A recent *in vivo* microdialysis study from the same laboratory emphasized the role of ACh and dramatically diminished the importance of Glu following sarin or VX exposure (O'Donnell et al., in press). Nonetheless, the current view is that the suppression of seizures via an inhibition of Glu release is of the utmost importance.

2. Antiglutamatergic drugs in the treatment of OP poisoning

The involvement of the cholinergic system in OP intoxication is well established and thus, the need for an anticholinergic drug(s) and enzyme reactivator(s) is unchallenged. Although the participation of the glutamatergic system in this process is also a given, the importance of an antiglutamatergic component in the treatment paradigm is still under debate. The report by Braitman and Sparenborg (1989) mentioned above showed that a high dose of MK-801 (5 mg/kg) completely blocked soman-induced seizure activity. Animals treated with MK-801 recovered faster and had a much greater probability of survival for 48 h after soman exposure than did controls. Exposing guinea pigs to a lethal dose of soman (2× LD_{50}) and applying thienylcyclohexylpiperidine (TCP), a non-competitive NMDA antagonist post-exposure, revealed that paroxysmal activity ceased in 10–20 min and all the animals survived (Carpentier et al., 2001). The paradigm consisted of a pretreatment with pyridostigmine and a combination of atropine and TCP arrested seizures and provided marked protection against morphological alterations and behavioral dysfunction. Notably, another report on the benefits of TCP found that although this compound demonstrated considerable protective potency, minor deficits in learning and memory were evident (de Groot et al., 2001). The neuroprotective effects of HU-211 (dexanabinol), a synthetic non-psychotropic analogue of tetrahydrocannabinol, on brain damage resulting from soman-induced seizures were examined in rats (Filbert et al., 1999). HU-211 is also a NMDA-receptor antagonist