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Therapeutic and reactivating efficacy of oximes K027 and K203 against a direct acetylcholinesterase inhibitor

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ABSTRACT

As oxime-based structures are the only causal antidotes to organophosphate (OP)-inhibited acetylcholinesterase (AChE), the majority of studies on these have been directed towards their synthesis and testing. In this study, experimental bispyridinium oximes K027 and K203, which have shown promising results in the last decade of research, were examined *in vivo* for their therapeutic and reactivating ability in acute poisoning by the direct AChE-inhibitor dichlorvos (DDVP), used as a dimethyl OP structural model. Additionally, the efficacy of oximes K027 and K203 was compared with the efficacy of four oximes (pralidoxime, trimedoxime, obidoxime and HI-6), already used in efficacy experiments and human medicine. To evaluate therapeutic efficacy, groups of Wistar rats were treated with equitoxic doses of oximes (5% LD_{50}, i.m.) and/or atropine (10 mg/kg, i.m.) immediately after s.c. DDVP challenge (4–6 doses). Using the same antidotal protocol, AChE activity was measured in erythrocytes, diaphragm and brain 60 min after s.c. DDVP exposure (75% LD_{50}). The oxime K027 was the most efficacious in reducing the DDVP induced lethal effect in rats, while the oxime K203 was more efficacious than trimedoxime, pralidoxime and HI-6. Significant reactivation of DDVP inhibited AChE was achieved only with oxime K027 or its combination with atropine in erythocytes and the diaphragm. Moreover, the acute i.m. toxicity of oxime K027 in rats was lower than all other tested oximes. The results of this study support previous studies considering the oxime K027 as a promising experimental oxime structure for further testing against structurally-different OP compounds.

1. Introduction

Highly toxic organophosphorus compounds (OPCs) are widely used as insecticides, but also include nerve poisons which are among the most toxic group of chemical warfare agents.

Organophosphorus compounds share a common mode of action (Mileson et al., 1998) exerting their toxic effects primarily via inhibition of acetylcholinesterase (AChE, EC 3.1.1.7), an enzyme responsible for hydrolysis of neurotransmitter acetylcholine in muscular and neural synaptic clefts (Eyer, 2003; Pope et al., 2005). Consequently, accumulation of acetylcholine causes excessive stimulation of central and peripheral muscarinic and nicotinic receptors, resulting in the complex clinical picture of cholinergic crisis.

Standard antidotal treatment of organophosphate poisonings consists of a muscarinic receptor antagonist (e.g. atropine), which counteracts the excessive muscarinic stimulation and reactivator of inhibited AChE (pyridinium oxime), which restores the physiological function of AChE. However, both experimental results and clinical findings have demonstrated insufficient and/or unequal efficacy of oximes against poisonings caused by structurally-different OPCs (Antonijevic and Stojiljkovic, 2007; Worek and Thiermann, 2013). Most oximes are sufficiently effective to reactivate sarin- or VX-inhibited AChE, whereas their potency to reactivate soman- or tabun-inhibited AChE is generally low (Dawson, 1994; Kassa et al., 2008, 2007). HI-6 has been demonstrated to be an effective antidote against soman, but ineffective against tabun poisoning (Kuča et al., 2009; Lundy et al.,

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2011). Trimedoxime and obidoxime have shown better efficacy in poisoning with OP insecticides, rather than the widely used pralidoxime (Antonijevic and Stojiljkovic, 2007).

Since the introduction of pralidoxime in 1955 (Childs et al., 1955), trimedoxime in 1958 (Poziomek et al., 1958), obidoxime and HI-6 in the late 1960s (Hagedorn et al., 1969; Luettringhaus and Hagedorn, 1964), numerous attempts have been made to improve the antidotal properties of the conventional mono- and bispyridinium oximes by modifying their structures. An additional approach has been to develop reactivators of butyrylcholinesterase (BChE, EC 3.1.1.8) as a 'stoichiometric OP bioscavenger' capable of neutralizing OP rapidly before it reaches physiologically important target sites, such as synaptic AChE (Radic et al., 2013). Finally, novel efforts to improve the antidotal treatment of OP poisonings have been undertaken through synthesis of non-oxime reactivators of inhibited AChE (Bhattacharjee et al., 2012).

As oxime-based structures are the only causal antidotes to OP-inhibited AChE, the majority of studies have been directed towards their synthesis and testing. During the last decade, a series of so-called K-oximes has been synthesized by researches from Czech Republic (Kuča et al., 2003a; Musilek et al., 2011a, 2007b). Among these experimental oximes, the oxime K203, having partial structural similarity to the oximes HI-6 and obidoxime, was shown to be effective against tabun poisoning (Kassa et al., 2008; Kovarik et al., 2009) and the oxime K027, having partial structural similarity to the oximes HI-6 and trimedoxime, was shown to be effective against a few pesticide poisonings (Kuca et al., 2010). Hence, oximes K203 and K027 have become promising candidates for further experimental testing to achieve higher efficacy and/or a broader spectrum of activities compared with the presently-available oximes.

Thus, our study was undertaken to investigate *in vivo* therapeutic and reactivating efficacies of oximes K027 and K203 in acute poisoning by the directly-acting AChE-inhibitor dichlorvos (DDVP), used as a dimethyl OP structural model, which according to our knowledge, has not been used in testing oximes K203 and K027, so far. Additionally, the efficacy of oximes K027 and K203 was compared with the efficacy of four oximes (pralidoxime, trimedoxime, obidoxime and HI-6) traditionally used in OPC poisonings (Table 1).

2. Materials and methods

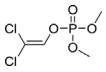
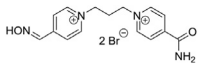
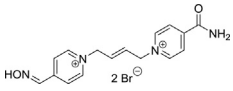
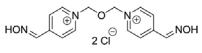
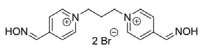
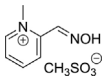
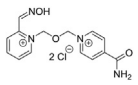
2.1. Animals

Male albino Wistar rats weighing 200–230 g were purchased from the Military Medical Academy, Belgrade, Serbia. They were housed in polycarbonate cages ($425 \times 266 \times 185\ mm^3$, maximum five rats per cage) in climate- and access-controlled rooms ($22 \pm 2\ ^\circ C$ and $55 \pm 10\%$ relative humidity). The day/night cycle was 12/12 h. Food and tap water were available *ad libitum*. The food was standard maintenance chow for rats purchased from The Veterinary Institute Subotica (Subotica, Serbia). Experimental animals were handled under the supervision of the Ethical Committee of the University of Belgrade, Faculty of Pharmacy (Serbia). All experimental procedures were conducted in accordance with the Animal Welfare Act of the Republic of Serbia (Official Gazette of the Republic of Serbia No. 41/2009), Directive 2010/63/EU on the Protection of Animals Used for Scientific Purposes and the Guide for the Care and Use of Laboratory Animals (National Research Council 2011, USA).

2.2. Chemicals

Dichlorvos (DDVP, *O,O*-dimethyl-*O*-2, 2-dichlorovinyl phosphate) was obtained as a gift from Chemical Agrosava d.o.o.

Table 1
Chemical structures, names and molecular weights of OP insecticide dichlorvos and tested oximes.

Chemical structure	Chemical name	Molecular weight (g/mol)
	**dichlorvos (DDVP)** *O,O*-dimethyl-*O*-2,2-dichlorovinylphosphate	220.98
	**K027** 1-(4-hydroxyiminomethylpyridinium)-3-(4-carbamoylpyridinium)–propane dibromide	446.14
	**K203** ((*E*)-1-(4-Carbamoylpyridinium)-4-(4 hydroxyiminomethylpyridinium)-but-2-ene dibromide	458.15
	**obidoxime (LüH-6)** 1,1'- oxydimethylene-bis-(4-hydroxyiminomethylpyridinium) dichloride	359.21
	**trimedoxime (TMB-4)** 1, 3-bis (4-hydroxyiminomethylpyridinium)-propane dibromide	443.98
	**pralidoxime (2-PAM)** 2-((hydroxyimino)-methyl)-1- methylpyridinium mesylate	232.26
	**asoxime (HI-6)** 1-(2-hydroxyiminomethylpyridinium)-3-(4-carbamoylpyridinium)-2-oxapropane dichloride monohydrate	377.22