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A comprehensive evaluation of the efficacy of leading oxime therapies in guinea pigs exposed to organophosphorus chemical warfare agents or pesticides

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ABSTRACT

The currently fielded pre-hospital therapeutic regimen for the treatment of organophosphorus (OP) poisoning in the United States (U.S.) is the administration of atropine in combination with an oxime antidote (2-PAM Cl) to reactivate inhibited acetylcholinesterase (AChE). Depending on clinical symptoms, an anticonvulsant, e.g., diazepam, may also be administered. Unfortunately, 2-PAM Cl does not offer sufficient protection across the range of OP threat agents, and there is some question as to whether it is the most effective oxime compound available. The objective of the present study is to identify an oxime antidote, under standardized and comparable conditions, that offers protection at the FDA approved human equivalent dose (HED) of 2-PAM Cl against tabun (GA), sarin (GB), soman (GD), cyclosarin (GF), and VX, and the pesticides paraoxon, chlorpyrifos oxon, and phorate oxon. Male Hartley guinea pigs were subcutaneously challenged with a lethal level of OP and treated at approximately 1 min post challenge with atropine followed by equimolar oxime therapy (2-PAM Cl, HI-6 DMS, obidoxime Cl_2, TMB-4, MMB4-DMS, HLö-7 DMS, MINA, and RS194B) or therapeutic-index (TI) level therapy (HI-6 DMS, MMB4-DMS, MINA, and RS194B). Clinical signs of toxicity were observed for 24 h post challenge and blood cholinesterase [AChE and butyrylcholinesterase (BChE)] activity was analyzed utilizing a modified Ellman's method. When the oxime is standardized against the HED of 2-PAM Cl for guinea pigs, the evidence from clinical observations, lethality, quality of life (QOL) scores, and cholinesterase reactivation rates across all OPs indicated that MMB4 DMS and HLö-7 DMS were the two most consistently efficacious oximes.

Introduction

Organophosphorus (OP) compounds, including pesticides and chemical warfare nerve agents (CWNAs), represent a threat to the general population, not only as possible weapons of terrorism (Okumura et al., 2005; Zurer, 1998; Hubbard et al., 2013; Baker, 2013; Dolgin, 2013), but also as chemicals that could be released from transportation and storage facilities during industrial accidents. Given the rapid onset of symptoms and toxicity of OP nerve agents, a quick-acting therapeutic regimen that is efficacious over the broad spectrum of OPs is needed. To provide the most effective therapy, medical countermeasures must be administered as soon as possible post-exposure.

The current U.S. therapy regimen includes the administration of atropine in combination with the oxime acetylcholinesterase (AChE) reactivator pralidoxime chloride (2-PAM Cl) (Inchem.org, 1989, 1999), followed by the anticonvulsant diazepam depending on whether convulsive symptoms are observed. This approach is accomplished with the use of the DuoDote® autoinjector kit (Meridian Medical

Abbreviations: 2-PAM Cl, pralidoxime chloride; AChE, acetylcholinesterase; BChE, butyrylcholinesterase; BBB, blood brain barrier; ChE, cholinesterase; CNS, central nervous system; CWNA, chemical weapon nerve agent; FDA, Food and Drug Administration; HI-6 DMS, 1-(((4-(aminocarbonyl) pyridinio)methoxy) methyl)-2-((hydroxyimino)methyl) pyridinium dimethanesulfonate; HLö-7 DMS, pyridinium,1-(((4-(aminocarbonyl) pyridinio)methoxy)methyl)-2,4-bis((hydroxyimino)methyl), dimesylate; HPLC, high performance liquid chromatography; IM, Intramuscular; LD_{50}, median lethal dose; MINA, monoisonitrosoacetone; MMB4 DMS, methoxime dimethanesulfonate, 1,1-methylene bis(4(hydroxyimino-methyl)pyridinium) dimethanesulfonate; MW, molecular weight; OP, organophosphorus; PR, protective ratio; QOL, quality of life; RA_{AChE}, baseline-normalized activity of acetylcholinesterase in peripheral blood; RA_{BChE}, baseline-normalized activity of butyrylcholinesterase in peripheral blood; RS194B, N-(2-(azepan-1-yl)ethyl)-2-(hydroxyimino)acetamide; SC, subcutaneous; TMB-4, trimedoxime bromide, TMB-4, 1′-propane-1,3-diylbis{4-[(E)-(hydroxyimino)methyl]pyridinium} dibromide; USDHHS, U.S. Department of Health and Human Services.

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Technologies™, Columbia, MD; https://www.duodote.com/meridian.aspx#) by trained emergency medical services personnel. The DuoDote® is a two-chambered, self-propelled syringe used for the intramuscular (IM) injection of atropine (2.1 mg free base) and 2-PAM Cl (600 mg) through the same needle.

Although the current treatment approach does protect against some OP toxicities, this protection does not extend across all OP CWNAs, i.e., it is not a broad-spectrum antidote (Worek and Thiermann, 2013; Thiermann et al., 2013). Unfortunately, when OP pesticides are included as potential intoxicants, the spectrum of therapeutic effectiveness is even less. Additionally, most leading oxime reactivators currently available worldwide, including 2-PAM Cl, are ionized with a hydrophilic bis-pyridinium quaternary backbone. As such, they are capable of reactivating cholinesterases (ChEs) in peripheral tissues, but not in the central nervous system (CNS) because they do not readily cross the blood brain barrier (BBB) (Voicu et al., 2013; Shih et al., 2012). Consequently, more effective oxime therapies, including a broader spectrum of activity and/or the capacity to cross the BBB, are being investigated to identify a more effective treatment than 2-PAM Cl. As the only true antidote, i.e., one that reactivates the target molecule AChE, a better oxime therapy would improve the nation's medical response capabilities.

While many oxime compounds have already been synthesized and tested for broad-spectrum efficacy (Bajgar, 2010; Shih et al., 2009; Voicu et al., 2013; Worek et al., 2007) as well as BBB penetration capabilities (Sit et al., 2011; Radić et al., 2012), an actual head-to-head and rigorous comparison of efficacy entailing quality of life (QOL) evaluation after treatment, peripheral blood cholinesterase reactivation, and lethality endpoints has been absent. The few studies to assess comparative efficacy in animals have typically been confined only to oximes within the same chemical class or moiety developed within a particular laboratory, rather than what is currently approved and fielded worldwide. Since those studies are also often conducted under non-standardized experimental conditions and lack other methodological controls to increase scientific rigor, the unintentional introduction of bias remains a possibility when interpreting the results.

The currently fielded oximes 2-PAM Cl (USA, UK, France), obidoxime Cl_2 (LüH-6; Germany, Netherlands), TMB-4 (trimedoxime bromide; Israel), and HI-6 DMS (Canada, Sweden) are efficacious against specific OP CWNAs (Antonijevic and Stojiljkovic, 2007; Bajgar, 2004, 2009, 2010; Cabal et al., 2004; Calic et al., 2006; Delfino et al., 2009; Eyer et al., 2008; Kassa, 1998, 2002, 2005; Kuca et al., 2007a, 2009; Lundy et al., 2006). Although there are few studies assessing the efficacies of oximes against OP pesticides, obidoxime Cl_2 is currently regarded as the most efficacious against pesticides (Worek et al., 2007). The search for a centrally acting oxime to maintain brain AChE activity has produced MINA and RS194B. MINA is a relatively small (molecular weight, or MW = 87.1 Da) AChE reactivator that has been shown to improve survivability against GB (Rutland, 1958; Askew, 1956; Dultz et al., 1957; Myers, 1959; Shih et al., 2009, 2010, 2012). RS194B (MW = 213.3 Da) and has been shown to reactivate human AChE in vitro and protect mice against VX, GB, and paraoxon (Radić et al., 2012).

HLö-7, HI-6, and obidoxime are bis-pyridinium oximes, each containing two charged pyridine rings (requisite in an oxime for optimal reactivation of VX-inhibited AChE; Esposito et al., 2014) joined by a dimethyl ether ($-CH_2-O-CH_2-$) linker (Fig. 1). The structural differences among HLö-7, HI-6, and obidoxime are in the number and position(s) of aldoximes on the pyridine rings (Kuca et al., 2006; Ekström et al., 2009). Also, one of the ring groups in HLö-7 and HI-6 is an isonicotinamide, which was included in their original synthesis to reduce toxicity (Oldiges and Schoene, 1970) but which, as molecular dynamic studies suggest, may also enhance ChE reactivation (Maxwell et al., 2008). HLö-7 and obidoxime are the most potent reactivators of phosphonylated and phosphorylated AChE, respectively (Worek et al., 2004). MMB4 and TMB-4 are the same 4-position bis-pyridinium aldoxime, except that MMB4 has a $-CH_2-$ linker while TMB-4 (a dibromide salt) has a $-C_3H_6-$ linker. TMB-4 originated in 1958 and was the first bis-pyridinium oxime to be effective against GA (Schoene and Oldiges, 1973; Inns and Leadbeater, 1983). The difference between these two similar compounds in terms of toxicity to the Hartley guinea pig by IM injection is remarkable: the 24-hour LD_{50} (median lethal dose) is 679 mg/kg (1514 μmol/kg) for MMB4 DMS (unpublished data), and 80 mg/kg (179 μmol/kg) for TMB-4 (Shih et al., 2009).

The overall objective of this study was to compare rigorously the efficacy of currently fielded and select promising novel AChE oxime reactivators under strict standardized experimental conditions to enable an accurate and unbiased assessment of their efficacies against OP CWNAs and pesticides. To accomplish this, the human equivalent FDA-approved dose of 2-PAM Cl was used as the experimental standard and the equimolar oxime therapy was administered to atropinized guinea pigs after an LD_{85} challenge of each OP CWNA or pesticides (data not shown). The LD_{85} was selected as the challenge level across OPs because it maximized the power of the test to discriminate among the oximes in terms of lethality. Additionally, those oximes with a safety index greater than 2-PAM Cl, i.e., MMB4 DMS, HI-6 DMS, MINA, and RS194B were also evaluated at an additional 'therapeutic dose' level equal to the median lethal dose (LD50) for the oxime divided by the TI for 2-PAM Cl. Overall efficacy was determined specifically in terms of QOL, blood cholinesterase levels in 24-hour survivors, and lethality.

Materials and methods

Organophosphorus compounds. The five CWNAs evaluated were tabun (GA; O-ethyl N,N-dimethyl phosphoramidocyanidate), sarin (GB; O-isopropyl methylphosphonofluoridate), soman (GD; O-pinacolyl methylphosphonofluoridate), cyclosarin (GF, cyclohexyl methylphosphonofluoridate), and VX (O-ethyl S-(2-diisopropylaminoethyl) methylphosphonothiolate). They were obtained from the U.S. Army Edgewood Chemical Biological Center (Aberdeen Proving Ground, MD). The purity values of the CWNAs were >98.5% as determined by gas chromatography. Chlorpyrifos oxon (purity ≥98%) and paraoxon (purity ≥98%) were purchased from Chem Service, Inc, West Chester, PA. Phorate oxon (purity ≥97.1%) was synthesized at Battelle's Analytical Chemistry Development Department (Columbus, OH). GB, GD, GF, and VX were diluted in 0.9% saline; GA and phorate oxon were diluted in multisol (a biocompatible solution of 48.5% water, 40% propylene glycol, 10% ethanol, and 1.5% benzyl alcohol, all v/v); and chlorpyrifos oxon and paraoxon were diluted in ethanol (99.96%), with the dosing solution concentration of each pesticide being limited to that which would allow the total volume of ethanol injected to be no more than 0.06% (v/w) of the body mass.

Oximes. 2-PAM Cl (pralidoxime chloride, 2-hydroxyiminomethyl-1-methylpyridinium chloride; supplied as an injectable drug at 100 mg/mL) and MMB4 DMS (methoxime dimethanesulfonate; 1,1-methylene bis[4(hydroxyimino) methyl]pyridinium) dimethanesulfonate; purity 100%) were supplied by the U.S. Department of Defense. HI-6 DMS (4-carbamoyl-1-[({2-[(E)-(hydroxyimino) methyl)]pyridinium-1-yl}methoxyl)methyl] pyridinium dimesylate; purity 98.7%), MINA ((1E)-1-(hydroxyimino)propan-2-one; purity 98.7%), TMB-4 (trimedoxime bromide; 1′-propane-1,3-diylbis{4-[(E)-(hydroxyimino)methyl]pyridinium} dibromide; purity 98.5%), and HLö-7 DMS (pyridinium,1-(((4-(aminocarbonyl) pyridinio)methoxy) methyl)-2,4-bis((hydroxyimino)methyl), dimesylate; purity 96.73%) were procured from Southwest Research Institute, San Antonio, TX. RS194B (N-(2-(azepan-1-yl)ethyl)-2-(hydroxyimino)acetamide; purity 96 ± 2%) was procured from Skaggs School of Pharmacy & Pharmaceutical Sciences (University of California, San Diego). Obidoxime Cl_2, LüH-6 (oxo-[[1-[[4-(oxoazaniumylmethylidene)pyridin-1-yl] methoxymethyl]pyridin-4-ylidene]methyl]azanium dichloride; purity 97.1%) was procured from Sigma Aldrich. MMB4 DMS, HI-6 DMS,