Monitoring the Pharmacokinetics of Pyridinium Aldoximes in the Body

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Abstract: A huge number of organophosphate poisonings occurring in agriculture, and a constant threat of misapplication of organophosphates as warfare agents require antidotes that efficiently improve the health-condition of intoxicated subjects.

Pyridinium aldoximes are medically used to reactivate the cholinesterase enzymes inhibited by organophosphates.

This paper outlines pharmacokinetics, metabolic disposition and blood-brain-barrier penetration of pyridinium aldoximes into the human and animal body, and the methods of their pharmacological analysis.

Key Words: Pyridinium aldoximes, pralidoxime, K-27, K-48, K-203.

INTRODUCTION

Acetylcholine (ACh) is a quaternary amine *neurotransmitter* in various organisms including humans. Its function is vital in both the *peripheral nervous* system (PNS), and the *central nervous system* (CNS) and *autonomic nervous system* (ANS). ACh is also the neurotransmitter in all *autonomic ganglia as well*. Acetylcholinesterases (AChEs) terminate the physical presence and functions of ACh at the junctions of the cholinergic nerve endings and their postsynaptic sites and effector organs. Synthetic cholinesterase inhibitors (anticholinesterases) have had a long line of history [1]. Anticholinesterases (e.g. cholinesterase blockers) cause accumulation of ACh at the vicinity of nerve terminals, so generate excessive overstimulation of cholinergic receptors through the central and peripheral nervous system. However, certain anticholinesterases are widely used, and among others have been approved for the treatment of Alzheimer's disease.

Organophosphates and organophosphonates (Table **1**) are inhibitors of cholinesterase enzymes [2-4]. Essential outcome of organophosphate penetration through the skin or through any other biological membranes results in severe poisoning of human beings and animals. Acute poisoning by anticholinesterase compounds such as sarin and organophosphate pesticides shows a wide range of signs and symptoms such as abdominal cramps; agitation, anxiety; coma; confusion; convulsion; bradycardia; arrhythmias; hypotension; blurred vision; lacrimation; miosis; mydriasis; increased salivation; perspiration; increased frequency in urination; urinary incontinence; diarrhea; nausea; vomiting; bronchoconstriction; increased bronchial secretion; rhinorrhea; depression of circulatory centers; hallucination; lethargy; seizures; somnolence and many others. These problems are due to the overabundance of acetylcholine in the central nervous system, parasympathetic nerve endings, somatic nerves and the ganglionic synapses of autonomic ganglia. Organophosphates covalently bind to the serine active site of AChE (Fig. **1**).

Even the phosphorylated esteratic site of AChE may undergo hydrolytic processes; the rate of regeneration is slow or negligible. Eyer [5] estimated the number of intoxications with organophosphorous pesticides as high as 3,000,000 per year, with the number of deaths and casualties about 300,000 per year. The "sarin-attack at the Tokyo Metro" resulted in a heavy 12 death toll in addition to the over 5000 injured persons [6,7]. Cholinesterase reactivators are used to counteract the anticholinesterases. The discovery of pralidoxime (**15**) [8] opened the way for the use of pyridinium aldoximes in the treatment of poisonings caused by organophosphorous compounds. Pralidoxime (**15**), the classical pyridinium aldoxime, become a special standard with therapeutic use. Pralidoxime (**15**) is a mono-pyridinium-mono-aldoxime. Its wide therapeutic use [2-4, 9] led to the synthesis of several novel pyridinium aldoxime compounds, some of them are bis-pyridinium-bis-aldoximes (obidoxime (**16**), HLö-7 (**21**), methoxime (**17**) and trimedoxime (**18**)), or HI-6 (**20**) is a bis-pyridinium-mono-aldoxime. The newly developed bis-pyridinium mono-aldoximes (certain K- compounds) will definitely improve the treatment of victims. The names, chemical structures, formulas, molecular sizes (Da) and the calculated logP values of some pyridinium aldoximes are given in Table **2**.

In the prevention of possible organophosphate poisoning (eg. for the rescueing stuff) pre-treatment by the antidote become also a requirement. As an alternative to pyridinium aldoximes administration of weak cholinesterase inhibitors [10-16] was also suggested. Another alternative possibility is offered by the administration of liposomes encapsulating the proper antidote [17].

Features and shortcomings of the therapy are summarized below.

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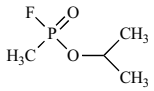
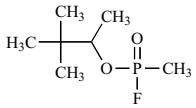
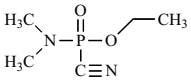
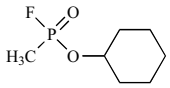
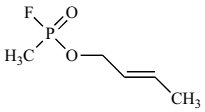
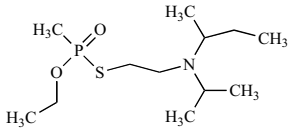
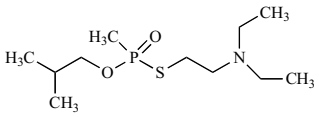
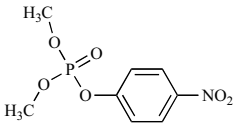
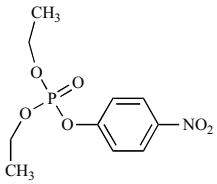
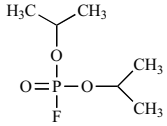
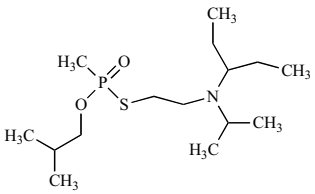
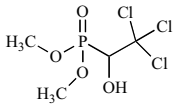
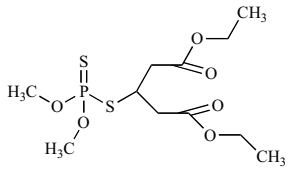
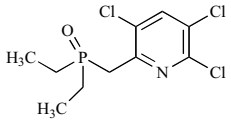
Table 1. The Chemical Structures, Molecular Sizes and Calculated logP Values of Several Organophosphorous Compounds, logP is Calculated Using a Special Sum of $logP_{Rekker}$, $logP_{annlogP}$, $logP_{annlogP2005}$. and $logP_{annlogP2006}$

No.	Name	Structure	Formula MW	logP
(**1**)	Sarin		$C_4H_{10}O_2FP$ 140.11	0.68
(**2**)	Soman		$C_7H_{16}O_2FP$ 182.20	1.75
(**3**)	Tabun		$C_5H_{11}N_2O_2P$ 162.15	-0.02
(**4**)	Cyclosarin		$C_7H_{14}O_2FP$ 180.18	1.46
(**5**)	Crotylsarin		$C_5H_{10}O_2FP$ 152.12	1.03
(**6**)	VX		$C_{12}H_{28}NO_2PS$ 281.44	2.44
(**7**)	VR		$C_{11}H_{26}NO_2PS$ 267.412	2.16
(**8**)	Methylparaoxon		$C_8H_{10}NO_6P$ 247.16	1.48
(**9**)	Ethylparaoxon		$C_{11}H_{16}NO_5P$ 273.25	2.16
(**10**)	DFP		$C_6H_{14}O_3FP$ 184.17	1.08* (given by $logP_{Rekker}$, only)
(**11**)	Russian VX		$C_{15}H_{34}NO_2PS$ 323.53	3.71

(Table 1. Contd….)

No.	Name	Structure	Formula MW	logP
(**12**)	Trichlorofon		$C_4H_8OPCl_3$ 257.44	0.70
(**13**)	Malathion		$C_{11}H_{21}O_6PS_2$ 344.41	2.15
(**14**)	Chlorpyrophos		$C_{10}H_{13}NOPCL_3$ 300.56	2.79

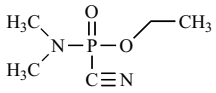
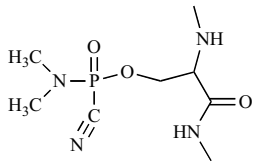
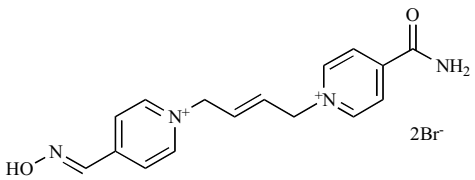
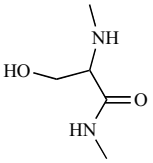
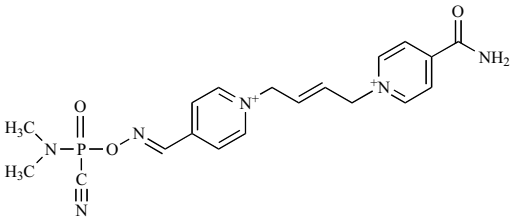
No.	Name	Structure	AnnlogP
	Tabun (**3**)		0.27
Step 1	Serin active site of acetyl-cholinesterase conjugated with tabun (**28**)		
	K-203 (**27**)		-3.27
Step 2	Serin active site freed from the nerve agent (**29**)		
Step 3	Conjugate of K-203 and tabun (**30**)		-3.75

Fig. (1). Three steps outlining interactions between serine active site – tabun – K-203.

1. Conjugation of serine active site by tabun
2. Serine active site freed from tabun
3. Conjugation of tabun by K-203.

PYRIDINIUM ALDOXIMES USED TO REGENERATE THE ACETYLCHOLINESTERASE ENZYME

Becker *et al*. [18,19] used spectrophotometry to monitor the direct reaction of different pyridinium aldoximes with certain organophosphorous compounds. Comparing the reactions [18], the highest rates were found for soman (**2**) with obidoxime (**16**), for sarin (**1**) with 2-PAM (**15**), and for tabun (**3**) with HI-6 (**20**). The reaction velocity decreased in the order of crotylsarin (**5**) **>** cyclosarin (**4**) **>** VX (**6**) [19]. The highest reaction rate was found for crotylsarin (**5**) and cyclosarin (**4**) with obidoxime. The reaction rate between nerve agents and 2-PAM (**15**) was lower than that of the bis-pyridinium oximes (obidoxime (**16**) and HI-6 (**20**)). Pyridinium aldoximes are used to reactivate phosphorylated AChEs after inactivation has taken place. Preventive treatment may be even more effective to prevent the enzyme from actions of organophosphorous compounds (mainly used as pesticides and warfare agents). The restoring mechanism is based on the direct reaction between organophosphate and pyridinium aldoxime. Certain bis-pyridinium aldoximes (such as obidoxime (**16**), methoxime (**17**), trimedoxime (**18**), HLö-7 (**21**)) comprised two aldoximes in one molecule. Intensive studies on chemical structure versus biological activity do not prove the advantages of –bis- aldoximes, the recent trend prefers bis-pyridinium mono aldoximes. Kuca *et al*. have synthesized a large number of pyridinium aldoximes. They also published methods to estimate the efficacy [20-22], as well as the lipophilicity of certain pyridinium aldoximes [23]. Csermely *et al*. [24,25] developed a method to determine the hydrophilic/lipophilic characteristics of some pyridinium aldoximes using thin-layer chromatography (TLC). The generally used reversed-phase TLC failed to give any information, as pralidoxime (**15**), obidoxime (**16**), K-27 (**22**) and K-48 (**24**) were absorbed at the start, they did not migrate from the site of their load. Utilizable results were obtained by using straight-phase TLC on plain silica, where obidoxime (**16**), K-27 (**22**) and K-48 (**24**) (the bis-pyridinium aldoximes) were found highly hydrophilic by their nature. Pralidoxime (**15**) was also found to be hydrophilic, but the numerical values for its slope and $R_{M,0}$ mirrored the mono-pyridinium aldoxime. Blood-brain (BBB) penetration of pyridinium aldoximes (both mono-pyridinium and bis-pyridinium compounds) is not in line with their calculated logP values when the PrologP program is used [26,27]. The exception is when the carrier-mediated and/or active-transport-facilitated processes have been supposed.

Corvino *et al*. [28] found adequate stability of pralidoxime (**15**) in solutions formulated for either intravenous or intramuscular administration. Pralidoxime (**15**) content remained higher than 90%, and retained sterility at environmental temperature for (at least) 28 days. Paddle and Dowling [29] determined the active ingredients in aqueous solutions. When atropine sulphate was the sole dissolved component, its content definitely decreased in a sealed ampoule kept at 80 °C. The degradation was essentially limited by the presence of any of either pralidoxime (**15**), or obidoxime (**16**), or HI-6 (**20**) in solution at pH 3 through 4. The higher pH or the absence of pyridinium aldoxime may result in base-catalized hydrolysis. Long-lasting autoclaving or excessive heating results in hydrolysis of pyridinium aldoximes (e.g. pralidoxime (**15**), obidoxime (**16**), HI-6 (**20**)). Acid amide and/or carboxilic acid derivative of the parent compound were identified. Other experiments found isonicotinamide degradation product of obidoxime (**16**) and HI-6 (**20**) after several days at 80 °C, while pyridine-4-aldoxime appeared as a minor breakdown product of obidoxime.

OVERVIEW OF THE ACTIONS OF PYRIDINIUM ALDOXIMES

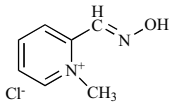
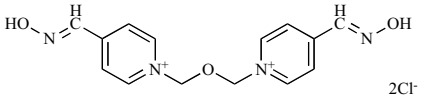
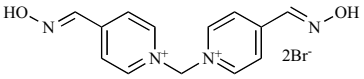
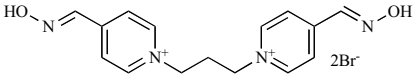
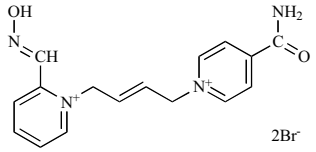
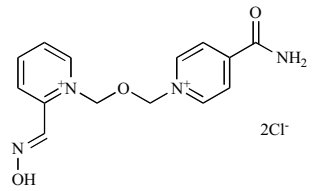
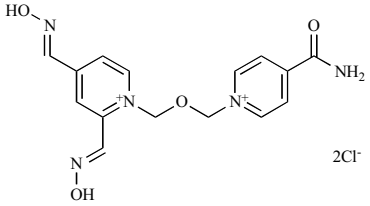
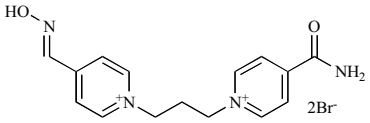
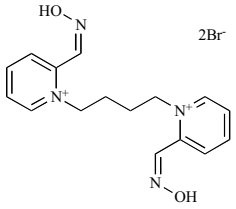
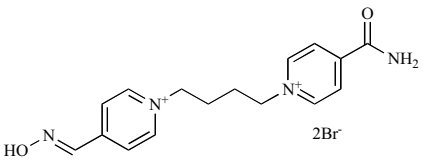
Antidote treatment of victims is possible, the therapy is known by the acronym: "A FLOP" (atropine, fluid, oxygen and pralidoxime (**15**)). The cholinesterase reactivating power of pralidoxime (**15**), obidoxime (**16**) and the other widely used official antidotes has been evaluated in depth in the basic text books of pharmacology [4] and toxicology [2,3]. Bajgar *et al*. [30,31] evaluated the value of treatment in reactivation of brain cholinesterases poisoned by cyclosarin (**4**), and Kuca *et al*. [32-34] outlined the structural requirements for reactivators.

An accidental injection to a U.S. Air Force aviator by an autoinjector happened. The military person recovered well, and no specific treatment was needed [35].

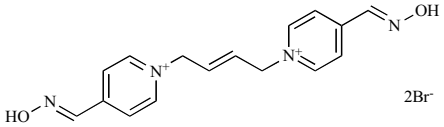
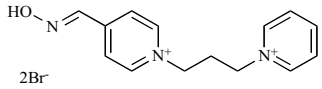
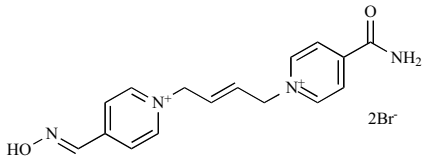
Shortcomings on the therapeutic value of certain pyridinium aldoximes has been studied by Kiderlen *et al*. [36]. The formation-activity-stability analysis showed that the diethylphosphoryl-obidoxime complex (DEPOB) is formed from obidoxime (**16**) and the organophosphate of diethylphosphorylated acetylcholinesterase. The generated DEPOB complex, however, has high anticholinesterase activity as a consequence of its decomposition products, such as the intermediate nitrile and cyanide. Experimental results also resulted in formaldehyde formation as reaction product of either O-demethylation or P-demethylation. Worek *et al*. [37] compared reactivation kinetics of acetylcholinesterase from different species following inhibition for 15 min using sarin (**1**), cyclosarin (**4**) and VX (**6**). Reactivation ability of oximes (2-PAM (**15**), obidoxime (**16**), HI 6 (**20**) and HLö-7 (**21**)) was characterized by using K_D, k_r and k_{r2}, the reactivation rate constant of the inhibited enzyme by aldoxime, the rate constant of displacement from the enzyme and specific reactivity of aldoxime to the phosphorylated enzyme, respectively. Worek *et al*. [37] found species differences among the reactivation kinetics. Worek *et al*. [38] expanded their former study [36] on cholinesterase reactivation. Reactivation rate constants of inhibited human AChEs were calculated for the relation of various organophosphates (VX (**6**), sarin (**1**), VR (**7**), cyclosarin (**4**), tabun (**3**), methyl-paraoxon (**8**), ethyl-paraoxon (**9**)) and pyridinium aldoximes (2-PAM (**15**), obidoxime (**16**), HI-6 (**20**), HLö-7 (**21**)). 2-PAM (**15**) was found to be a relatively weak reactivator of organophosphate inhibited AChE, while obidoxime (**16**) was effective against the majority of nerve agents (sarin (**1**), VX (**6**), VR (**7**)). Soman (**2**) was an exception from the easily possible reactivation procedures due to rapid aging of soman-AChE complex.

Wolthuis *et al*. [39] developed an isolated organ technique. Their method is based on the fact that the victims of poisoning by either organophosphorous pesticides or nerve agents are usually deceased due to respiratory failure. Seeger *et al*. [40] suggested the evaluation of oxime efficacy in

Table 2. The Chemical Structures, Molecular Sizes and Calculated logP Values of Several Pyridium Aldoximes, logP is Calculated Using a Special Sum of $logP_{Rekker}$, $logP_{annlogP}$, $logP_{annlogP2005}$ and $logP_{annlogP2006}$

No.	Name	Structure	Formula MW	logP
(**15**)	Pralidoxime (2-PAM)		$C_7H_9N_2O$ 137.18	-2.56
(**16**)	Obidoxime		$C_{14}H_{16}N_4O_3$ 288.34	-2.87
(**17**)	Methoxime		$C_{13}H_{14}N_4O_2$ 288.34	-2.74
(**18**)	Trimedoxime		$C_{15}H_{18}N_4O_2$ 258.31	2.45
(**19**)	BI-6		$C_{16}H_{18}N_4O_2$ 298.38	-3.04
(**20**)	HI-6		$C_{14}H_{16}N_4O_3$ 288.34	-3.20
(**21**)	HLö-7		$C_{17}H_{21}N_5O_2$ 327.43	-2.62
(**22**)	K-27		$C_{15}H_{18}N_4O_2$ 286.37	-2.84
(**23**)	K-33		$C_{16}H_{120}N_4O_2$ 300.40	-2.62
(**24**)	K-48		$C_{16}H_{20}N_4O_2$ 300.40	-2.79

(Table 2. Contd….)

No.	Name	Structure	Formula MW	logP
(**25**)	K-75		$C_{16}H_{18}N_4O_2$ 298.38	-2.46
(**26**)	K-156		$C_{14}H_{17}N_3O$ 243.34	-2.75
(**27**)	K-203		$C_{16}H_{18}N_4O_2$ 298.38	-3.04

organophosphate (OP) -poisoning using a modification of the classical indirect field stimulation technique. Muscle force generation by indirect stimulation was practically completely blocked during 1 micromol/L paraoxon circumfusion for 20 min, and was restored using circumfusion of 1 micromol/L obidoxime for 20 min. Worek *et al*. [41] tested classical pyridinium aldoximes using human erythrocyte AChE to eliminate the so called species dependence of the experiments. Comparisons were done in the effects and interactions of different organophosphates, organophosphonates and phosphoramidates, as well as pralidoxime (**15**), obidoxime (**16**), HI-6 (**20**), HLö-7 (**21**). Reactivation of AchE was practically resistant to the effect of obidoxime (**16**), HI-6 (**20**), HLö-7 (**21**) were determined following phosphorylation with phosphoramidates, but phosphonylated AchE was easily reactivated following inhibition. Phosphonylated AChE showed the best reactivation by HLö-7 (**21**), while obidoxime (**16**) was the most potent to reactivate AChE inhibited by organophosphates. Thiermann *et al*. [42] outlined the complex monitoring of victims including the red blood cell acetylcholinesterase activity (RBC-AChEA) of the poisoned subjects. When RBC-AChEA was restored by the oxime therapy to become 30%, or higher, neuromuscular transmission can be supported using relatively low atropine doses, e.g. several milligrams.

The effects of pyridinium aldoximes are shown using poisoned animals as real model for future treatments. Thompson *et al*. [43] summarized the therapeutic dosage of pralidoxime chloride to be used in the treatment of organophosphate poisoning. The recommended dose was a 1 g bolus plus continuous infusion with 0.5 g/h that maintains blood pralidoxime (**15**) level > 4 μg/mL. Poisindex [44] recommended a 1 g intermittent intravenous infusion to adults twice a day. Cassel *et al*. [45] used Wistar rats to follow how HI-6 (**20**) concentration change in blood and brain following soman-intoxication. 90 μg/kg dose using s.c. administration of soman was applied. The strong inhibition of acetylcholinestarase activity was found in soman (**2**) intoxicated animals (blood, striatum, hippocampus, cerebellum, hypothalamus, cortex, medulla/pons, alike), that was partially counter balanced by 50 to 100 mg/kg i.m. dose of HI-6 (**20**). Krummer *et al*. [46] found *in vitro* an equipotent cholinesterase reactivation ability of HI-6-2Cl and HI-6-DMS, although HI-6-DMS has better dissolution properties when used in a dry/wet autoinjector [47]. Cabal *et al*. [48] made specification of the strucures of oximes from the point of view of *in vitro* reactivation of tabun-inhibited AChEs. Their results mirrored the superiority of trimedoxime (**18**) over K-27 (**22**), however, K-203 (**27**) was not mentioned in their publication. When the effects of various pyridinium aldoximes were checked using the „survival in rats exposed to the oraganophosphate paraoxon model" K-27 (**22**) and K-48 (**24**) showed superiority over trimedoxime (**18**) [49]. Some recently developed pyridinium aldoximes were evaluated by Kuca *et al*. [50, 51]. The therapeutic and reactivation value of K-203 (**27**) was markedly better, than that of the commercially available oximes (obidoxime (**16**), trimedoxime (**18**) and HI-6 (**20**)), while K-156 (**26**) does not improve the pharmacologically important parameters in tabun-poisoned rats and mice [50,51].

Recently, Three Basic Lines of Tests have been Used

1. Petroianu's group carried out their *in vitro* [14, 52-55] and *in vivo* [56-63] experiments to counterbalance both the insecticide poisoned human red blood cells (*in vitro* tests) and rats (*in vivo* experiments). Petroianu and Kalász [64] made comparison of protective effect of various pyridinium aldoximes to reactivate cholinesterases. The compounds were ranked their effects based on the relative risk of death/survival rate of oranophosphate exposed rats compared to the rats untreated by aldoxime. The best to least effective order was found such as K-27 (**22**), K-48 (**24**), methoxime (**17**), HI-6 (**20**), pralidoxime (**15**), K-33 (**23**). Petroianu and Lorke [65] have evaluated the predictive value of *in-vitro* testing for *in-vivo* efficacy by summarizing and comparing determination of enzyme reactivation capacity (tan α), lipophilicity (in silico estimation of logP), to the *in vivo* protective effects in diisopropyl-fluorophosphate (**10**) (DFP) poisoning (reduction of relative risk of death after DFP (**10**) poisoning) and toxicity (LD_{50}). *In vitro* reactivation capacity of human red blood cell acetylcholinesterase activity was not

found to correlate for *in vivo* efficay in rats following DFP (**10**) exposure.

2. Kuca *et al*. worked with models based on the treatments of warfare agents [21, 48, 50, 51, 66-131], traced the effect of various bis-pyridinium aldoximes in the case of tabun (**3**) [21, 48, 50, 51, 66, 67, 72, 79, 82, 87, 91, 99, 100, 102, 103, 109, 110, 112, 113-116, 123-126, 128, 129, 131], sarin (**1**) [72, 75, 77, 78, 80, 107, 119, 120, 132], cyclosarin (**4**) [70-72, 78, 83, 89, 92, 104-106, 113, 114, 116, 118, 126, 130], soman (**2**) [87, 116, 125, 126], VX (**6**) [94, 96], Russian VX (**11**) [69, 94, 101, 122], in addition to paraoxon (**9**) [20, 22, 100, 109, 114, 115, 123, 129, 133], DFP (**10**) [22], trichlorfon (**12**) [134], chlorpyrifos (**14**) [86, 88, 97, 106, 122], and malathion (**13**) [135] poisoned animals, tissues and enzyme preparations. They used both *in vivo* test and *in vitro* reactivation of red blood cell cholinesterases.

3. A recent method uses reactivation of tabun-inhibited pure acetylcholinesterase enzyme [66]. The method can be used for *in vitro* screening, then the best reactivators are subjected to further studies *in vivo*. The gross changes in the neurotoxic markers deal with an additional way of evaluation [66]. The markers cover a wide scale of signs and sympthoms, such as posture, muscular tone, lacrimation, salivation, nose secretion, rearing, urination, defecation, tremors, clonic movements, ataxia, activity, approach response, touch response, pupil size, etc. K-75 (**25**) showed the highest potency to reduce the lethal toxicity of tabun-induced neurotoxic markers, while HI-6 (**20**) and obidoxime (**16**) were shown less effective than the average.

Petroianu *et al*. [53, 54, 56, 60, 136, 137] and Kuca *et al*. [21, 22, 50, 51, 68, 70-72, 74, 75, 78-80, 82, 83, 85, 86, 87-89, 90, 92, 93, 95, 96, 99-102, 104, 105, 109, 110- 116, 118-120, 123-126, 128-135, 138, 139] made comparison between the efficiency of the currently used and new bis-pyridinium aldoxime type cholinesterase reactivators following their developments.

PHARMACOKINETICS

Discovery of Wilson and Ginsburg [8] directed the attention to pralidoxime as a powerful reactivator of alkylphosphate inhibited acetylcholinesterase. Pharmacokinetics of pralidoxime (**15**) were soon outlined by characterizing its absorption [140], distribution [141, 142] and elimination [140, 141, 143, 144]. The fate of radiolabelled 2-PAM in the poisoned rat's body was studied by Uehara *et al*. [145]. Intravenous administration of ^{14}C-2-PAM resulted in its minor ratio in the brain. On the contrary, intramedullary injection provided 72-90% of ^{14}C-2-PAM for the brain in its unchanged form, with a 1.52 h half life. ^{14}C-2-PAM was excreted in the urine and faeces either after i.v. or intramedullary administration. Srivastava and Malik [146] followed the time course of plasma level, disposition kinetics, and the dosage regimen for pralidoxime (**15**) administered to healthy male buffalo calves following the administration of 15 mg/kg and 30 mg/kg intramuscular dosage. Apparent volume of distribution, absorption half life, elimination half life and the total body clearance were 0.83 – 1.01 L/kg, 1.08 ± 0.19 h, 3.14-3.19 h and 184.9-252.1 mL/kg/h, respectively. A plasma level of ≥ 4 μg/mL was maintained for up to either 4 or 6 h when using either 15 mg/kg or 30 mg/kg doses, respectively. The suggested dosage regimen [146] of 2-PAM (**15**) in the treatment of organophosphate poisoning in buffaloes would be 25 mg/kg followed by 22 mg/kg at 8 h intervals.

Holland and Parkes [142] suggested the human use of 500 mg 2-PAM (**15**) dose with intramuscular administration of instead of i.v. in case of emergency. The same i.m. dose can also be used prophylactically before crop spraying with organophosphorous AChEIs. Holland *et al*. [147] compared the rate of uptake of both 750 mg and 500 mg pralidoxime mesilate given alone and also together with 2.0 mg atropine sulphate intramuscularly to forty-four healthy volunteers. No significant difference in the uptake rate of 2-PAM (**15**) was found when 2-PAM (**15**) was administered alone or in combination with atropine. On the contrary, intravenously administered thiamine hydrochloride decreased 2-PAM (**15**) excretion in the first three hours, the plasma half life was risen together with the plasma level of 2-PAM (**15**), and the elimination rate constant dropped. Josselson and Sidell [148] suggested either competition of thiamine and 2-PAM (**15**) for the common renal secretory mechanism, or thiamine alters the membrane transport of 2-PAM (**15**).

Pharmacokinetics of pralidoxime (**15**) was extensively studied by Sidell *et al*. [148-154]. 2-PAM (**15**) was either i.v. [148-151, 154] or p.o. [152, 153] administered to control (that is: healthy, non-poisoned) subjects. 2-PAM (**15**) dose was about 5 mg/kg (3 through 10 mg/kg). The counterion was either chloride, or methanesulfonate, 2-PAM-Cl was i.v. administered, while both 2-PAM-Cl and 2-PAM methanesulfonate was taken orally. Pharmacokinetics of pralidoxime fits a two compartment open model. 2-PAM (**15**) is widely distributed in the body, rapidly eliminated by the kidney. The elimination is supposedly done through secretion by renal tubules as its urinary clearance exceeds that of creatinine simultaneously determined.

About 75 min (between 66 and 79) $t_{½}$ was found following i.v. injections, while p.o. administration resulted in practically doubled period of $t_{½}$.

Willems *et al*. [155] applied pralidoxime methylsulphate to treat patients with poisoning of ethyl parathion, methyl parathion, dimethoate and bromophos. 4.42 mg/kg loading dose was followed by a maintenance dose of 2.14 mg/kg/h, and the pralidoxime (**15**) level was determined. The mean pralidoxime (**15**) level varied between 2.12 and 9 mg/L. Pharmacokinetic data, such as volume of distribution, elimination half life and the total body clearance were 2.77 ± 1.45 L/kg, 3.44 ± 0.99 h and 0.57 ± 0.27 L/kg/h, respectively. Houzé *et al*. [156] monitored pralidoxime (**15**) level of a patient of acute organophosphate poisoning and having initial atropine therapy. The aim was to keep the presumed therapeutic concentration, and also to monitor the urinary output, the method was HPLC with electrochemical detection. The loading dose was 400 mg pralidoxime mesilate, using slow perfusion in 30 min, followed by 100 mg/kg over 18 h, 200 mg/kg over 3 days, and 100 mg/kg over 60 h. The pralidoxime (**15**) concentration in serum and in urine was in the range of 2.1 through 5.6 μg/ml and 810 through 2727 μg/ml, respectively. Renal clearance of pralidoxime (**15**) was

also calculated (0.5 through 075 L/kg/h). Houzé *et al*. [156] supposed pralidoxime metabolism was the difference of infused and excreted drug. Medicis *et al*. [157] scrutinized the then usually recommended 1 g adult dose of pralidoxime (**15**) administered in a 15-30 min infusion. Their computer-predicted pharmacokinetics warned that the possible concentration would fall below 4 mg/L in a relatively short period (within one hour and a half). They [157] recommended the same dose of pralidoxime (**15**) infused with a far a longer time frame, such as 16 mg/kg i.v. infusion over 30 minutes followed by 3.2 mg/kg for 3.75 h. The loading dose followed by the continouos infusion resulted in therapeutic levels for 257.3 ± 50.5 minutes, while the earlier used method did it for 118.1 ± 52.1 minutes. Schexnayder *et al*. [158] monitored pralidoxime (**15**) treatment with continuous infusion for 12-43 hours. The patients were children and adolescents having been subjected to organophosphate poisoning. The steady state concentration, volume of distribution, elimination half life and clearance were 22.2 ± 12.3 mg/L, 1.7 through 13.8 L/kg (the higher values applied to severly poisoned patients), 3.6 ± 0.8 hours and 0.88 ± 0.55 L/h/kg. Schexnayder *et al*. [158] suggested a loading dose of 25 mg/kg through 50 mg/kg that can be followed by using a continuous infusion of 10 through 20 mg/kg/h.

Pharmacokinetics of obidoxime (**16**) therapy was followed by Thiermann *et al*. [159] in organophosphate poisoned patients. They agreed on the administration of obidoxime (**16**) as an i.v. bolus (250 mg) followed by continuous infusion (750 mg/24h). Reactivation period was found longer in organophosphate poisoned patients than expected based on *in vitro* experiments. Reactivation of AChE enzyme by obidoxime (**16**) was nearly complete when its administration started in 1 hour, however, obidoxime (**16**) was practically ineffective in oxydemethonmethyl poisoning when the therapy started 1 day after ingestion. Humans [160-163], marmoset monkeys [164], swines [165-167], guinea pigs [164,165,168], dogs [169, 170], rats [68,171-173], rats [45, 174] and mice [174] have been treated using either HI-6 dichloride (HI-6-2-Cl) and HI-6-DMS, or both of them. Cassel *et al*. [45] used Wistar rats to follow how HI-6 (**20**) concentration change in blood and brain following soman-intoxication. Brain microdialysis was done in the striatum, blood microdialysis was arranged in the jugular vein. Control blood samples were taken from a tail vein. The trait of HI-6 (**20**) brain concentration showed a definite delay, including the t_{max} and $t_{½}$ values, while soman (**2**) pretreatment did not influence these pharmacokinetic parameters.

Garrigue *et al*. [175] studied the metabolism and disposition of pyrimidoxime and HI-6 (**20**). Both compounds were labeled with ^{14}C on the oxime group. They compared the elimination of these compounds in the urine,and found that 85% of radioactivity was eliminated in the urine in 24 hours. Lundy *et al*. [165] followed the pharmacokinetics of HI-6 (**20**) using HPLC. The subjects were both swines and guinea pigs, both intravenous (i.v.) and intramuscular (i.m.) administrations were performed, and both HI-6-2Cl and HI-6 dimethanesulphonate (HI-6-DMS) were administered. The results indicated in both species the similarity of the three pharmacokinetic phases for HI-6-2Cl with i.m. administration, the absorption, distribution and elimination giving $t_{½}$ of 2.00, 12.59 and 81.45 minutes, respectively. The C_{max} (maximum of concentration, μg/mL), t_{max} (time required to reach the maximum of concentration, min), AUC_{inf}, (area under the concentration versus time curve to infinite time, μg/mL min), V_D (volume of distribution, L/kg) and CL (clearance, mL/min/kg) values were determined as 60.13 ± 3.34, 9.17 ± 1.54, 4457 ± 632, 0.53 ± 0.07 and 5.10 ± 0.90, respectively. Administration of HI-6-DMS resulted in very similar characteristics, except an almost doubled $t_{½}$ of distribution phase, while its AUC_{inf} was about 20% higher (5131 instead of 4457). The authors stated thereby that equimolar dose of HI-6-2Cl and HI-6-DMS displayed an identical pharmacokinetic profile. Tekes *et al*. [176, 177] determined plasma concentration of K-27 (**22**). A simple and reliable HPLC method was developed. The injected sample size was 20 μL, following the standard clean-up procedure. Octyl silica stationary phase was applied (Supelcosil LC8, 250 mm x 4.6 mm, i.d.), and the mobile phase consisted of 8% of methanol and 92% of aqueous buffer of 15 mM phosphate buffer at pH 2.6, 1 μM 1-octanesulfonic acid sodium salt.The chromatograms were obtained at thermostated room temperature (26 °C), detection was done at 286 nm. One major peak represented the K-27 (**22**), its homogeneity was controlled using HPLC-MS. Pharmacokinetic parameters of K-27 (**22**) were determined following both intraperitoneal (i.p.) and intramuscular (i.m.) administrations. The time course of K-27 (**22**) was approximated after i.m. administration. The equation of a straight line was found between 15 and 120 minutes, that is:

$$y = yo + ax = 195 – 1.16\ x$$

meaning the tentative 0 min concentration as high as 195 μg/mL. Similar pharmacokinetics was found by Kalász *et al*. [178] for K-48 (**24**) following both i.m. and i.p. administrations. Roberts and Buckley [179] definitely suggested considering the pharmacokinetic and pharmacodynamic principles at the management of patients exposed to poison. This principle includes the changes of pharmacokinetic parameters in patients with acute poisoning. Absorption kinetics, volume of distribution, elimination half-life, and elimination may be changed, especially when the patient is subjected to a high dose of poison.

ANALYSIS FOR PHARMACOKINETICS

The change in the chemical structure of any drug takes place either by metabolism or degradation. Rubnov *et al*. [180] outlined the acid-catalysed autocatalytic way of obidoxime (**16**) degradation. They also estimated that the shelf-life of pralidoxime (**15**) is more than 37 years (the t_{90}, or shelf life is the time by which 10% of the drug has been degraded). To establish pharmacokinetics requires proper analytical methods. HPLC separation with ultraviolet monitoring is the standard method when the serum level is monitored.

Sakurada *et al*. [172] used an excellent mobile phase for separation of pralidoxime (**15**) from both serum and brain samples. The eluent was composed of 0.2 M phosphoric acid also containing 2 mM sodium 1-octanesulfonate and 0.1 M diethylamine. Cassel *et al*. [45] determined HI-6 (**20**) level using RP-HPLC, the elution was monitored at 295 nm. Dopamine level in the rat brain was also monitored by RP-

HPLC, however, detection was done using an electrochemical detector. High sensitivity determinaton of pyridinium aldoximes from either rat brain, or from any rat brain segment may require sophisticated monitoring, such as using either electrochemical detection [156, 177] or hyphenated mass spectrometry (for review, see Csermely *et al*. [181]). The calibration curves were linear in each case. Ultraviolet detection makes reliable analysis possible through the range between 1 and several hundred μg/mL of plasma covering the first 4 hours following either i.m. or i.p. administration. Electrochemical analysis of pyridinium aldoximes from the brain, and especially from brain segments requires a much more sensitive method than the detection of UV absorbance.

Clean-up of serum samples may be circumvented by the use of capillary electrophoresis [182, 183].

METABOLIC DISPOSITION OF PYRIDINIUM ALDOXIMES

Way and Way [184] summarized the results of the research of metabolic pattern of pralidoxime (**15**). The used method was mainly paper chromatography, however, Amberlite CG-50 cation exchange column was also used [185] to separate the parent 2-PAM (**15**) from its two metabolites:1-methyl-2-cyanopyridinium ion and 1-methyl-2-methoxypyridinium ion. Ligtenstein *et al*. [186] isolated and identified two HI-6 (**20**) metabolites from rat urine using preparative HPLC followed by mass spectrometry, infrared- and nuclear magnetic resonance spectroscopy. Both metabolites contained 2-pyridone moiety, one of them with an intact pyridinium aldoxime structural element. Morgan *et al*. [187] reported that trimedoxime (**18**) was metabolized on one of the aldoxime groups. The metabolic procedure went through the nitrile intermediate to a carbamido compound.

Benkő *et al*. [188] made microsomal treatment of K-27 (**22**) and K-48 (**24**), the two bis-pyridinium mono aldoxime compounds. While K-27 (**22**) showed definite resistancy to the treatment, K-48 (**24**) was digested. Further analysis of Benkő *et al*. [189] indicated formation of a hydoxylated metabolite of K-48 (**24**) on the alkyl chain bridge. *In vivo* metabolic study of Benkő *et al*. [189] did not show any metabolite of K-48 (**24**) in the rat serum and cerebrospinal fluid. However, urinary elimination of an epoxydated K-48 (**24**) on the connecting alkyl chain was detected.

BLOOD-BRAIN-BARRIER PENETRATION OF PYRIDINIUM ALDOXIMES

The brain capillary endothelial cells are connected by tight cell membrane junctions forming a continuous cell membrane practically impermeable to hydrophilic compounds [190-193] to the central nervous system. This barrier system is called blood-brain barrier (BBB), and provides protection for the brain from polar exogenous compounds, xenobiotics. Endogenous transporter proteins are embedded in the BBB that function to shuttle polar nutrients and certain other endogenous molecules into the CNS from the periphery. These active transport vector systems may be targeted for hydrophilic drug delivery into the brain in cases of neurological diseases and pathologies, such as irreversible inhibition of enzymes in the central nervous system. Paraquat is a double-charged bis-pyridinium compound. Shimizu *et al*. [194] described the carrier-mediated process in BBB penetration of paraquat, hypothesizing that the paraquat crosses the BBB by utilizing the neutral amino acid transport system (NAATS).

Geldenhuys *et al*. [195] traced the ability of choline transporter to facilitate numerous compounds to penetrate into the central nervous system. They made 3D-QSAR molecular modelling studies, comparative molecular field analysis (CoMFA) and comparative molecular similarity analysis (CoMSIA) to build their model. All compounds were energy minimized for the CoMFA and CoMSIA analyses, and fitted to the choline binding site constructed by using various interactions. Hydrogen bond interaction, bulk cavity methyl acceptor, ionic interaction and one more bulk cavity methyl acceptor were taken into consideration. Several compounds (mainly primary, tertiary and quaternary amines) were experimentally checked with tritiated choline uptake reduction by using *in situ* brain perfusion studies. Goldenhuys *et al*. [195] illustrated the validity of their model by the example how the cis-cyclic analogue of choline was well fitted into the favourable steric region of the choline transporter, whereas the trans-cyclic analogue was not. However, the only pyridinium compound (N-methyl pyridinium ion) did not fit properly to the model choline transporter. Zhang *et al*. [190] specified certain mono-pyridinium and bis-pyridinium compounds as novel high affinity ligands for the choline transporter of blood-brain barrier. The choline transporter has been found to be responsible for transporting some other highly polar compounds, such as quaternary ammonium ellipticine and a nitrogen mustard alkylating agent. Firemark *et al*. [196] followed penetration of pralidoxime into the brain by the help of radiolabelling (^{14}C-2-PAM)

Sakurada *et al*. [172] found direct proof that pralidoxime (**15**) is able to penetrate BBB. After intravenous doses to rats, 2-PAM (**15**) appeared in the microdialysate. The striatal extracellular/blood concentration ratio was about 0.1. Cassel *et al*. [45] demonstrated the penetration of i.m. injected HI-6 (**20**) into the brain, and they measured HI-6 (**20**) concentrations and effects in various brain segments. Lorke *et al*. [197] found less extent of penetration of K-27 (**22**) and K-48 (**24**) into the rat brain, than that of obidoxime (**16**). The entry of all three oximes into the brain was found minimal, and did not explain the better therapeutic efficacy of K-27 (**22**) and K-48 (**24**), compared to obidoxime (**16**).

To explain the definite penetration of pyridinium aldoximes through the BBB, there are three possibilities. Both of them show concentration limitation.

1. Geldenhuys *et al*. [195] experimentally prove the existance of facilitation of penetration of pyridinium aldoximes by the help of choline transporter.
2. Sakurada *et al*. [172] did not confirm the role of amino acid transporter in the BBB penetration of pralidoxime (**15**).
3. Gibbon and Way [185] formulated the possible tautomer forms of pralidoxime (**15**): the quaternary ammonium, zwitterionic pralidoxime, and the non-ionic fully conjugated formation. The non-ionic form is dominant at pH = 9 [185], however, its formation is initiated at any basic

pH condition, that is over pH 7.0. It is the pH at physiological condition. Drastic change in logP takes place in the cases of mono-pyridinium aldoximes and bis-pyridinium bis-aldoximes where the transient, fully conjugated forms exist. Similar redox system was suggested by Bodor *et al*. [198-200] that delivery of dihydropyridine form of quaternary pyridinium salt may facilitate the transport to and from the brain of mice.

Bis-pyridinium mono-aldoximes may not produce fully conjugated formation. As these two groups of pyridinium aldoximes do not show essential differences, their BBB penetration, the major BBB transporting force seems to be the choline transporter. Petroianu *et al*. [201] systematically investigated the effect of organophosphate paraoxon (**9**) on the brain penetration of pralidoxime. BBB penetration of pralidoxime (**15**) was determined without/with preliminary treatment of rats by using paraoxon, and without/with perfusion of the brain with isotonic saline. Brain pralidoxime of about 6 % was found at C_{max}, while AUC in the brain was 8% to 12%, in absence and presence of paraoxon (**9**) pretreatments, respectively. Lorke *et al*. [137, 201, 202,] systematically investigated the fact of pyridinium aldoxime penetration of BBB. They analyzed the brain structure, and the way of transport to the brain and to the cerebrospinal fluid. They found experimental proof of minimal effect of paraoxon (**9**) on pralidoxime (**15**) brain concentration when the subjects of treatment were rats. Ballantyne and Swanston [203] found the transient increases in intraocular tension of rabbits following intramuscular administration of pralidoxime mesylate, however, the increase in tension did not statistically correlate with plasma or aqueous humour pralidoxime (**15**) concentration.

CONCLUSIONS

Pralidoxime (**15**) is a pyridinium aldoxime, and there are several other classical pyridinium aldoximes (obidoxime (**16**), methoxime (**17**), trimedoxime (**18**), BI-6 (**19**), HI-6 (**20**), HLö-7 (**21**)). The generally used antidotes to organophosphate poisoning have been thought not to have the perfect beneficial effect [204, 205]. The range of "classical pyridinium aldoximes" has been completed by numerous newly synthesized promising compounds, which are mainly bis-pyridinium mono-aldoximes.

Alternatives to oxime therapy have been reviewed by Peter *et al*. [205] stated the fifty-year-old question: "Adjuncts and alternatives to oxime therapy in organophosphate poisoning – is there evidence of benefit in human poisoning?". There has been a relatively high morbidity and mortality with organophosphate poisoning despite the use of atropine (a specific antidote as antagonist on muscarinic ACh receptor) and pyridinium aldoximes to reactivate AChE. Adjunct/alternative treatments include topical application of creams to reduce poison absorption; haemoperfusion to enhance toxin elimination; administration of cholinesterase rich plasma; using an adenosine receptor agonist, or clonidine, diazepam, magnesium, N-acetyl cysteine to counteract poison effect. After reviewing the possible alternatives Peter *et al*. [205] have not found enough evidence to change the present therapy. Some of the newly synthesized bis-pyridinium mono-aldoximes offer the best hope for proper treatment, as it has been published by numerous papers. K-27 (**22**) and K-48 (**24**) were found eminent antidotes to methylparaoxon (**8**) and paraoxon (**9**), while K-203 (**27**) was a potent reactivator of tabun-inhibited acetylcholinesterases.

These K-compounds may have adequate concentration in the blood stream, and can penetrate to the central nervous system. Overdose of pyridinium aldoximes should be avoided, as their penetration probably has a certain carrier, or a transporter, the BBB transport is thereby limited [181, 206].

ACKNOWLEDGEMENTS

The research was financially supported by the grant of T049492 of OTKA (Hungarian National Granting Agency, Budapest, Hungary).

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Received: 03 November, 2008 Revised: 12 January, 2009 Accepted: 12 January, 2009