Review

PYRIDINIUM OXIMES: RATIONALE FOR THEIR SELECTION AS CAUSAL ANTIDOTES AGAINST ORGANOPHOSPHATE POISONINGS AND CURRENT SOLUTIONS FOR AUTO-INJECTORS

Miloš P. STOJILJKOVIĆ[1] and Milan JOKANOVIĆ[2]

Medical Department, Actavis Trading Ltd. Representative Office Belgrade[1], Galenika Institute[2], Belgrade, Serbia

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During the last five decades, five pyridinium oximes were found to be worthy of use as antidotes against nerve agents in humans: pralidoxime, in a form of chloride or PAM-2 Cl and mesylate or P2S (against sarin, cyclosarin and VX), trimedoxime or TMB-4 and obidoxime or LüH-6 (both against tabun, sarin and VX), HI-6 (against sarin, soman, cyclosarin and VX) and HLö-7 (against all the five nerve agents).
In order to provide the auto-injector with the best and most potent acetylcholinesterase reactivator, the Defence Research and Development Canada (DRDC) received in the 1990s a core funding from the federal government's CBRN research and Technology Initiative (CRTI). Its ultimate result should be three products: (1) 3-in-1 auto-injector (atropine, HI-6 dimethanesulphonate and avizafone, as anticonvulsant), (2) 2-in-1 auto-injector (atropine and HI-6 dimethanesulphonate) and (3) HI-6 dimethanesulphonate in a vial for administration by the medically trained personnel.
Previous experimental and clinical experience suggests that, among the oximes mentioned, only trimedoxime and obidoxime can be used for acetylcholinesterase reactivation and antidotal protection against most of the organophosphorus insecticides.
The search for an "omnipotent" oxime, effective in reactivation of AChE inhibited with both nerve agents and organophosphorus insecticides, is still ongoing.

KEY WORDS: *acetylcholinesterase, nerve agents, organophosphorus compounds, reactivation*

Atropine seemed to be the only antidote available for the treatment of poisoning with organophosphorus compounds (OPC) until 1951, when Jandorf made the first step towards the solution of this problem by showing that hydroxylamine could destroy OPCs *in vitro* (1). These results inspired Wilson to try adding hydroxylamine to tetraethyl pyrophosphate (TEPP)-inhibited acetylcholinesterase (AChE) *in vitro*, obtaining thus the first known successful reactivation of the previously irreversibly phosphorylated AChE (2).

In 1955 oximes were discovered as specific antidotes, being much more efficient than hydroxylamine (3, 4). Actually, pralidoxime (PAM-2), the first tested oxime, could reactivate the phosphorylated enzyme about a million times faster than hydroxylamine (5).

In the same year it was established that the enzyme did not remain susceptible to reactivation by the oximes forever – the enzyme-inhibitor complex could undergo an "ageing" reaction depending on the chemical structure of inhibitor (6). In 1959, the experiments on diisopropylfluorophosphate (DFP)-inhibited butyrylcholinesterase (BuChE) showed that the mechanism underlying this "ageing" process consisted of dealkylation of the OPC residue (7).

Soman is a nerve agent notorious for its rapid "ageing" properties; the half-time of this process is only 2.3 min in bovine erythrocyte AChE at 37 ºC and pH 7.35. Soman, in turn, has a branched and voluminous pinacolyl leaving group (Figure 1), rendering thus the nucleophilic attack by the oximes impossible (8). It was later confirmed that the "ageing" process is fastest after poisoning of rats with soman (half-time 4 min) and significantly slower after poisoning with the other three major nerve agents sarin (12 h), tabun (46 h) and VX (12 days) (9).

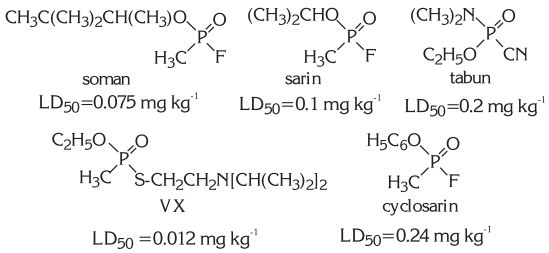

Figure 1 *Chemical structure of nerve agents and their acute toxicity after subcutaneous administration in rats (according to ref. no. 10)*

MECHANISM OF ACTION OF OXIMES

Oximes can bind reversibly to the AChE molecule either at its catalytic site (active centre), allosteric (peripheral) site or at both sites of the enzyme; binding to either site affects the catalytic properties of the AChE (cf. 11). Every oxime has a strong positive charge in its molecule that navigates it to the negatively charged anionic site at the active centre of AChE, attracting thus the oxime molecule closer to the molecule of the OP residuum (cf. 5). Thereafter, the oxime group performs the so-called nucleophilic attack at the phosphorus atom of the OP residuum, which results in the formation of an unstable enzyme-inhibitor-oxime complex. The ultimate result is the splitting of the complex into a phosphorylated oxime and reactivated enzyme (Figure 2).

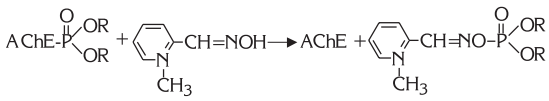

Figure 2 *Reactivation of phosphorylated acetylcholinesterase with pralidoxime and formation of reactivated enzyme and phosphorylated oxime*

CONTEMPORARY OXIMES

Among the many classes of oximes investigated so far, those that found clinical application can be divided into the monopyridinium and bispyridinium ones. The only currently used monopyridinium oxime is PAM-2, while the most significant bispyridinium oximes are trimedoxime (TMB-4), obidoxime (LüH-6, Toxogonin), HI-6, and HLö-7 (Figure 3) (12).

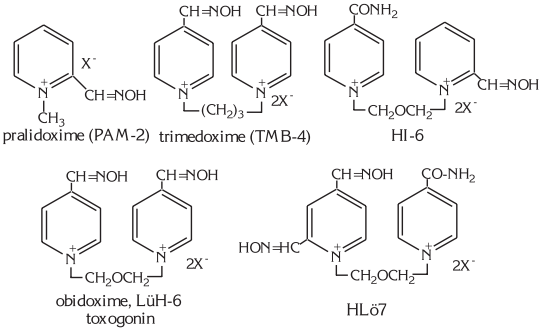

Figure 3 *Chemical structure of pyridinium oximes used in the treatment of OPC poisoning. X stands for an anion*

Pralidoxime (PAM-2)

Pralidoxime (pyridinium-2-aldoxime) was synthesised in the USA in 1955 (13). Its four salts, chloride (PAM-2 Cl), methiodide, methysulphate and mesylate (P2S), were investigated and introduced into practice (5). PAM-2 is very efficient in reactivating AChE inhibited with sarin or VX (14-19), but is not with tabun or soman (20-22).

As a quaternary pyridinium salt, PAM-2 does not readily penetrate the blood-brain barrier (BBB), and this is why pro-2-PAM was synthesised as a pro-drug of PAM-2 that can gain access to the central nervous system (CNS). Unexpectedly, it turned out to be even less effective than PAM-2 against experimental poisoning with paraoxon (23). However, it appears that in OPC poisoning PAM-2 can pass BBB at higher concentrations when given with atropine.

Trimedoxime (TMB-4)

Chemically, TMB-4 Cl_2 is a 1,3-bis(4-hydroxyiminomethyl-1-pyridinio)propane-dichloride and was synthesised in the USA in 1957 (24) and patent-protected in 1967 (25). It is the only of the major bispyridinium oximes with a propylene bridge between the two pyridinium rings. Experiments have shown that TMB-4 is a more potent reactivator of the DFP-inhib-

ited AChE than PAM-2 (26) and by 15 % to 40 % better reactivator than LüH-6 in case of tabun inhibition (27). TMB-4 was also the first oxime that was efficient in the treatment of animals intoxicated with tabun (28-30). It can also protect animals poisoned with sarin or VX, but not those poisoned with soman (29, 31). At the same time, TMB-4 is the most toxic oxime among the "great four"; it was shown in mice that its median lethal dose (LD_{50}) is 3, 4 and 8 times lower than respective LD_{50} of LüH-6, PAM-2 and HI-6 (32).

Obidoxime (LüH-6, Toxogonin)

Chemically, LüH-6 Cl_2 is a [1,3-bis(4-hydroxyimminomethyl-1-pyridinio)-2-oxapropane] dichloride. It was named in honour of A. Lüttringhaus and I. Hagedorn who synthesised it in Germany and introduced into medical practice in 1964 (33). The new oxime immediately showed a significant potential as antidote in OPC poisoning (34). Being a good AChE reactivator, LüH-6, administered *in vivo* with atropine, efficiently protected experimental animals against poisoning with tabun (20, 35), sarin (20, 35) and VX (35). LüH-6 was somewhat more efficient than TMB-4 as antidote against tabun poisoning (36). Nevertheless, like PAM-2 and TMB-4, LüH-6 was inefficient against soman poisoning in mice (29), guinea-pigs (20) and primates (37). In contrast to TMB-4, when administered with atropine in pyridostigmine-pretreated guinea-pigs, LüH-6 can give some protection against soman, as well (20). Although less toxic than TMB-4, this oxime can show its hepatotoxic potential (5, 38).

HI-6

The first oxime that could reactivate soman-inhibited AChE and provide at least some protection of animals experimentally poisoned with this nerve agent. It was synthesised in 1966 and given the code name HS-6, after the last name initials of Ilse Hagedorn and Klaus Schoene (39). Shortly thereafter its congener was launched; an oxime named HI-6 after the initials of the last name of Ilse Hagedorn and the first name of her student Irmo Stark, chemists who synthesised it in Freiburg, Germany in 1966 (9, 40). It was shown that HI-6 was more potent than LüH-6 and HS-6 in protecting various rodent species from poisoning with soman (17, 20, 41), as well as sarin and especially VX (17, 20, 29). The only drawback of HI-6 was that this oxime could not reactivate tabun-inhibited AChE (42, 43). As a consequence, it was proven to be inefficient when used alone against tabun poisoning (17, 20, 29). Nevertheless, in some other studies HI-6 showed a similar degree of protection against tabun and soman poisoning in primates (37), while sufficiently high doses of the oxime could even protect rats from multiple lethal doses of tabun (44). The toxicity of HI-6 is low, actually the lowest among the aforementioned oximes (29, 32, 45), with the LD_{50} in rats as high as 781.3 mg kg^{-1} or 2071 μmol kg^{-1} (12).

HLö-7

The fourth and the last important "Hagedorn oxime" (after LüH-6, HS-6 and HI-6) is HLö-7, named after Ilse Hagedorn and Marianne Löffler and also synthesised in Freiburg, Germany in 1986 (46). Chemically, this oxime is a 1-[[[4-(aminocarbonyl)pyridinio]methoxy]methyl]-2,4-bis[(hydroxyimino)methyl] pyridinium-diiodide. The new oxime reactivates AChE inhibited by any of the four major nerve agents (47-50), as well as the enzyme inhibited by cyclosarin (51). In addition, HLö-7 is more efficien than PAM-2, LüH-6 and HI-6 in restoring neuromuscular transmission impaired by *in vitro* superfusion of the neuro-muscular preparation with tabun, sarin, soman or cyclosarin (52). It was found that HLö-7 induced a significant reactivation of AChE in mice diaphragms previously inhibited with tabun, sarin, soman and cyclosarin (53). Although both HI-6 and HLö-7 can antagonise sarin-induced hypothermia (proving thus that they, when given with atropine, can pass the BBB and gain access to the CNS) (53, 54), the LD_{50} of HLö-7 is 2.5 times lower than of HI-6, which means that the new oxime is more toxic (53). The cardiovascular tolerability of HLö-7 was similar, but not as good as that of HI-6, at least in anaesthetised guinea-pigs (55). On the scale of efficacy, HLö-7 turned out to be somewhat more effective than HI-6 against tabun and VX poisoning and less effective against sarin, soman, and cyclosarin poisoning (51, 56). In guinea-pigs, a species considered the closest model to the primates in OPC poisoning, HLö-7-induced protection against tabun poisoning was significantly better than that of HI-6, while HI-6 was only slightly more efficient than HLö-7 in soman poisoning (57).

OXIME SELECTION CRITERIA

The main criteria to be used when choosing the best oxime against the four classical nerve agents

tabun, sarin, soman and VX, as well as cyclosarin, used by the former Iraqi regime (51) are:

A) ability to reactivate AChE inhibited by nerve agents *in vitro* and *in vivo*,
B) when given with atropine, ability to assure survival of experimental animals, poisoned with multiple lethal doses of nerve agents,
C) when given with atropine, ability to pass the BBB and reach the CNS in significant and therapeutically relevant concentrations,
D) ability to express some "direct pharmacological effects" such as influence on the liberation of acetylcholine from pre-synaptic nerve endings (58),
E) the oxime's own toxicity itself,
F) chemical and pharmaceutical stability of the oxime and
G) suitability for formulating the oxime in the form of auto-injector.

According to these criteria,

A) Only HLö-7 can reactivate AChE inhibited by all the nerve agents mentioned *in vitro* and *in vivo*. Follows HI-6, which cannot reactivate only tabun-inhibited AChE.
B) When administered with atropine, both HI-6 and HLö-7 can assure *in vivo* protection against multiple LD_{50}s of all five nerve agents, with HI-6 being more efficient with sarin, soman and cyclosarin and less efficient with tabun and VX. These differences seem not to be of clinical importance.
C) Although without carbamate pre-treatment none of the oximes studied in animals can induce a significant reactivation of brain AChE inhibited by soman, the fact that only HI-6 and HLö-7 can reactivate the brain AChE inhibited by sarin and antagonise sarin-induced central hypothermia, proves the ability of these two oximes to reach the brain at concentrations relevant to exert their central antidotal effects.
D) All of the oximes have some of the so-called "direct pharmacological (or non-AChE-reactivating) effects". However, it seems that HI-6 and especially HLö-7 have some pre-junctional properties that decrease the amount of acetylcholine released into the synaptic cleft, an effect so beneficial under the conditions of AChE inhibition by soman (58).
E) Among the five current oximes, HI-6 is by far the least toxic, followed by HLö-7, PAM-2, LüH-6 and TMB-4. A clinical study with HI-6 administered at doses up to 500 mg in 22 healthy volunteers revealed no adverse effects (59).
F) While older oximes PAM-2, TMB-4 and LüH-6 are stable in water solutions and can be prepared in ampoules, HI-6 and HLö-7 are not and therefore should be kept as powder over a longer time and dissolved immediately before use.
G) Both HI-6 and HLö-7 were successfully prepared in the form of wet-dry auto-injector. Similar devices containing PAM-2, TMB-4 and LüH-6 also exist, but in the form of solution with atropine (60).

MODERN AUTO-INJECTORS

Based on various doctrines, every country had its rationale while developing auto-injectors to protect their armed forces. Table 1 contains some of the solutions of eight countries, mostly belonging to NATO (61).

Table 1 *Prophylactic and treatment doctrines/auto-injector content in some countries (according to ref. no. 61)*

Country	Prophylaxis*	Anticholinergic	Oxime	Anticonvulsant
United Kingdom	pyridostigmine	atropine	P2S	avizafone
United States	pyridostigmine	atropine	PAM-2Cl	diazepam
Germany	pyridostigmine	atropine	LüH-6	-
Finland	pyridostigmine	atropine	LüH-6	-
Norway	pyridostigmine	atropine	LüH-6	diazepam
The Netherlands	pyridostigmine	atropine	LüH-6	avizafone
Sweden	pyridostigmine	atropine	HI-6	-
Canada	pyridostigmine	atropine	HI-6	diazepam

*Pyridostigmine pre-treatment is administered per os, one 30-mg-tablet every 8 hours

It is evident that all these armies favour the use of pyridostigmine as a pre-treatment antidote and atropine as classical antimuscarinic drug. They also have an oxime as a part of the antidotal combination, but the choice of the oxime varies a lot - from pralidoxime salts in the UK and the US to LüH-6 in Norway, Finland, Germany and The Netherlands and HI-6 in Sweden and Canada.

Due to significant costs, only a few of these countries provide anticonvulsants in the form of auto-injector. The greatest advantage of avizafone over diazepam is water solubility, which allows its presence in aqueous solutions of atropine as the "wet" part of the "wet-dry" autoinjectors (with HI-6 powder being the dry part).

CANADIAN HI-6 INITIATIVE

Various world armies have auto-injectors against nerve agents that contain atropine and one of the four classical oximes. The drawbacks of the current HI-6-based solutions include: (a) The lack of a commercial source of supply of Good Manufacturing Practice (GMP)-grade HI-6, (b) a cumbersome system of multiple auto-injectors and (c) an incomplete data package to support a regulatory submission (missing of the HI-6 Drug Master File) (62).

In order to provide the auto-injector with the best and most potent AChE reactivator, the Defence Research and Development Canada (DRDC) received in the 1990s a core funding from the federal government's CBRN Research and Technology Initiative (CRTI). DRDC will receive financial and scientific assistance through a six-nation collaboration (between Canada, Germany, the Netherlands, Norway, Sweden and the United Kingdom). Its ultimate result should be three products: (1) 3-in-1 auto-injector (atropine, HI-6 dimethanesulphonate and avizafone, as anticonvulsant), (2) 2-in-1 auto-injector (atropine and HI-6 dimethanesulphonate) and (3) HI-6 in a vial for administration by medically trained personnel.

In a recent study performed in anaesthetised pigs, HI-6 dimethanesulphonate was shown to have the same pharmacokinetic properties as the original HI-6 dichloride salt. In the same study, both HI-6 salts, administered with atropine, successfully protected guinea-pigs from poisoning against a fivefold LD_{50} of soman or cyclosarin (63).

This project continues to progress under a trilateral Memorandum of Understanding, signed by the governments of Canada, the United Kingdom and the Netherlands (62).

This initiative suggests that HI-6 has won the tight race with HLö-7 for the title of the best causal antidote against nerve agent poisoning. Even though HLö-7 has a few advantages over HI-6, it is quite obvious that in combination with atropine both oximes are superior to TMB-4, LüH-6 and especially to PAM-2 in the treatment of nerve agent poisoning.

Note that clinical experience with PAM-2 iodide used in combination with atropine and diazepam in the treatment of the Tokyo underground sarin attack victims in 1995 was highly favourable (64). This, however, does not mean that this oxime should be the drug of choice in the treatment of nerve agent poisonings, because it is less efficient against tabun and soman (65).

ORGANOPHOSPHORUS INSECTICIDES

Poisoning with OP insecticides (OPIs) yielded quite the opposite results. The worst were obtained with HI-6 and HLö-7 and, to some extent, with PAM-2, while the most efficient oximes were TMB-4 and LüH-6 (66, 67). Jokanović and Maksimović (66) studied acute oral toxicity of 26 OP insecticides in rats and the efficacy of antidotal treatment involving TMB-4, LüH-6, PAM-2 and HI-6 (given with atropine and diazepam) in animals dosed with 2 LD_{50}s of the insecticides. The success of the therapy depended on the chemical structure of OPC. The oximes were potent antidotes against insecticides having the phosphate structure, and provided some protection against phosphonates, phosphorothiolates, phosphorothionates, and phosphorodithioates. However, none of them were effective against dimethoate and pyridafenthion. TMB-4 turned out to be the most effective oxime in the treatment of OP insecticide poisoning. In addition, AChE inhibited with OP insecticides, having mainly dimethyl and diethyl phosphate structure, can spontaneously reactivate with half-times for dimethyl phosphates of 1.3 h *in vitro* and 2.1 h *in vivo*. The rate of spontaneous reactivation for diethyl phosphates bound to AChE is about ten times slower (68).

Although the only two randomised controlled clinical trials performed so far did not result in a final proof of the efficacy of oximes in the treatment of

OPI poisonings in humans (69), our experimental and clinical experience suggests that, among the oximes mentioned, only TMB-4 and LüH-6, relatively toxic themselves, can provide reactivation and antidotal protection against most OPIs.

When it comes to an ultimate choice of an oxime for this indication, it seems that

somewhat better tolerability and the existence of auto-injectors with atropine speak in favour of LüH-6 over TMB-4.

CONCLUSION

There is no universal oxime, capable of protecting against all the known OPCs. In military toxicology, HI-6 methansulphonate seems to be the best choice for nerve agent poisoning, and should make part of the wet-dry auto-injector with atropine and avizafone. On the other hand, LüH-6 dichloride, in ampoules or in auto-injectors, seems to be the best choice for the treatment of OPI poisoning. The best alternatives for the proposed solutions would be HLö-7 and TMB-4, respectively.

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Sažetak

PIRIDINIJEVI OKSIMI: RAZLOZI ZA NJIHOV ODABIR KAO KAUZALNIH ANTIDOTA PROTIV OTROVANJA ORGANOFOSFATIMA I SADAŠNJA RJEŠENJA ZA AUTOINJEKTORE

Tijekom proteklih pet desetljeća, za pet piridinijevih oksima ustanovljeno je da zavređuju da budu upotrijebljeni kod ljudi kao antidoti protiv živčanih bojnih otrova: pralidoksim, u obliku klorida, PAM-2 Cl ili mesilat, P2S (protiv sarina, ciklosarina i VX-otrova), trimedoksim ili TMB-4 i obidoksim ili LüH-6 (oba protiv tabuna, sarin i VX-otrova), HI-6 (protiv sarina, somana, ciklosarina i VX-otrova) i HLö-7 (protiv svih pet živčanih bojnih otrova).
Radi osiguranja autoinjektora s najboljim i najpotentnijim reaktivatorom acetilkolinesteraze, Kanadska uprava za obrambena istraživanja i razvoj (DRDC) primila je tijekom 1990-ih godina osnovnu financijsku potporu od Istraživačke i tehnološke inicijative u oblasti kemijske, biološke, radiološke i nuklearne zaštite (CRTI) Vlade Kanade. DRDC će primati financijsku i znanstvenu pomoć suradnjom šest zemalja (između Kanade, Njemačke, Nizozemske, Norveške, Švedske i Velike Britanije). Njezin bi krajnji rezultat trebala biti tri proizvoda: (1) "3-u-1" autoinjektor (atropin, HI-6 dimetansulfonat i avizafon, kao antikonvulzant), (2) "2-u-1" autoinjektor (atropin i HI-6 dimetansulfonat) i (3) HI-6 dimetansulfonat u bočici za primjenu od medicinski obrazovanog osoblja.
Prethodna eksperimentalna i klinička iskustva sugeriraju da, među spomenutim oksimima, jedino trimedoksim i obidoksim, koji su relativno toksični *per se*, mogu osigurati reaktivaciju acetilkolinesteraze i antidotsku zaštitu protiv većine organofosfornih insekticida.
Potraga za "omnipotentnim" oksimom, djelotvornim i protiv svih živčanih bojnih otrova i protiv svih organofosfornih insekticida, još je u toku.

KLJUČNE RIJEČI: *acetilkolinesteraza, organofosforni spojevi, reaktivacija, živčani bojni otrovi*

REQUESTS FOR REPRINTS:

Miloš P. Stojiljković
Actavis Trading Ltd. - Representative Office Belgrade
Kneginje Zorke 2, 11000 Belgrade, Serbia
E-mail: *mistojiljkovic@actavis.co.yu*