ORIGINAL ARTICLE

A COMPARISON OF THE THERAPEUTIC EFFICACY OF CONVENTIONAL AND MODERN OXIMES AGAINST SUPRALETHAL DOSES OF HIGHLY TOXIC ORGANOPHOSPHATES IN MICE

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Summary: 1. The therapeutic efficacy of various oximes (pralidoxime, obidoxime, methoxime, HI-6, HLö-7, BI-6) against supralethal nerve agent poisoning (soman, sarin, cyclosin) in mice was tested. 2. New oxime BI-6, synthesized in our laboratory, is significantly more efficacious than conventional oximes but a little less efficacious than other H-oximes (HI-6, HLö-7). 3. H-oximes (HI-6, HLö-7) seem to be the most efficacious reactivators of nerve agent-inhibited acetylcholinesterase for antidotal treatment of supralethal nerve agent poisoning in mice.

Key words: *Soman; Sarin; Cyclosin; Pralidoxime; Obidoxime; Methoxime; HI-6; HLö-7; BI-6; Mouse*

Introduction

The basic mechanism of action of the highly toxic nerve agents - irreversible inhibition of acetylcholinesterase (AChE, EC 3.1.1.7), that results in the accumulation of endogenous acetylcholine (ACh) in synaptic cleft and paralysis of nerve impulse transmission in the central and peripheral nervous system, is well known (1, 13). Nevertheless, there are still problems with the effective treatment of acute poisoning with them (2,5).

Conventional antidotal treatment of nerve agent poisoning consists of cholinolytic drugs (preferably atropine) to counteract the effects of accumulated ACh at the receptors and oximes (preferably pralidoxime and obidoxime) to reactivate nerve agent-inhibited AChE (5,13). Generally, conventional oximes (pralidoxime, obidoxime, methoxime) have been considered to be adequate against some nerve agents such as sarin and VX but rather ineffective against other nerve agents such as soman and cyclosin (11,15,16). The differences in the oxime effectiveness are mainly due to the variation in aging rates; the process by which nerve agent-inhibited AChE is converted to a form that cannot be reactivated by oximes (2,13). Soman-inhibited AChE cannot be reactivated within minutes following poisoning and therefore the treatment of soman poisoning is much more difficult than VX or sarin poisoning (2,9).

This fact led to the synthesis of a series of bisquaternary oximes, designated „H-oximes", that in combination with anticholinergic drugs have been relatively successful in antagonizing soman poisoning (9,15). Among the H-oximes, HI-6 and HLö-7 have been the most promising against nerve agent poisoning (8,14) and consequently have been the best studied. New asymetric bispyridinium oxime, synthesized in our laboratory, designated BI-6 1-[4-carbamoylpyridinium]-4-[2-hydroxyiminomethylpyridinium]-2-buten dibromide also belongs to H-oximes (3).

The purpose of this study was to compare the therapeutic effectiveness of new oxime BI-6 and other H-oximes as well as conventional oximes against supralethal poisoning with chosen nerve agents (soman, sarin, cyclosin) in mice.

Methods

Male mice (19 - 24g) obtained from Konárovice were housed in an air-conditioned room (20-22°C) on 12-h light/12-h dark cycles and were allowed access to food and tap water ad libitum. The principles of laboratory animal care were followed and the handling of animals was made under the supervision of the Ethics Committee of the Medical Faculty of Charles University and the Military Medical Academy (Hradec Králové).

To evaluate the maximal treatment efficacy of tested oximes, we have used their prophylactic administration (5 minutes before nerve agent). The prophylactic treatment should give better results than treatment after poisoning and reduces the nerve agent-specific influence of aging.

We determined the i.m. LD_{50} values of oximes in mice and i.m. oxime doses that are sufficient for the 50% survival of mice poisoned with supralethal doses ($2xLD_5$) of nerve agents (ED_{50} values). The values were estimated by probit analysis based on 24h mortality data in at least five

groups of six animals each (17). For ED_{50} determination, the oximes were administered i.m. in the same solution as atropine sulfate (21 mg/kg). The administration of atropine alone failed to prevent the mortality following exposure to all three nerve agents tested. In the end, the safety ratio (SR; LD_{50}/ED_{50}) of each oxime was evaluated.

Results

The LD_{50} values of all oximes tested are shown in Table 1. New oxime BI-6 is less toxic for mice than conventional oximes pralidoxime and obidoxime but significantly more toxic than other H oximes (HI-6, HLö-7).

Table 1: Toxicity parameters of oximes tested

OXIMES	LD_{50} (mg/kg)
Pralidoxime	230.5 (192.2 - 276.5)
Obidoxime	188.4 (156.3 - 208.0)
Methoxime	641.8 (590.5 - 716.0)
BI-6	266.3 (248.5 - 285.4)
HI-6	671.3 (627.4 - 718.3)
HLö-7	356.0 (293.0 - 431.0)

The ED_{50} and SR values of oximes tested are shown in Table 2 - 4. In the case of soman poisoning, there are big differences in the efficacy between conventional oximes and H oximes. While all conventional oximes (pralidoxime, obidoxime, methoxime) are practically ineffective against supralethal dose of soman (ED_{50} values are too high), H oximes are sufficiently effective in relatively small doses. The new oxime BI-6 is a little less effective than other H oximes (Table 2).

Table 2: Prophylactic antidotal potency (ED_{50}) and safety ratio (SR) in soman poisoned mice ($2xLD_{50}$)

OXIMES	ED_{50} (mg/kg)	SR (LD_{50}/ED_{50})
Pralidoxime	92.5 (81.4 - 105.1)	2.5
Obidoxime	> 120	< 1.6
Methoxime	105.7 (95.5 - 117.1)	6.1
BI-6	16.7 (10.4 - 26.7)	16.0
HI-6	3.5 (2.4 - 5.0)	192.9
HLö-7	3.3 (2.4 - 4.6)	106.9

In the case of sarin poisoning, practically all tested oximes are sufficiently effective against supralethal dose of sarin in relatively low doses. The oxime BI-6 is a little less effective than other H oximes again (Table 3).

In the case of cyclosin intoxication, the conventional oximes are significantly less effective than H oximes. Their ED_{50} values are relatively high although not so high as in the case of poisoning with soman. The oxime BI-6 seems to be as effective as other H oximes against cyclosin (Table 4).

Table 3: Prophylactic antidotal potency (ED_{50}) and safety ratio (SR) in sarin poisoned mice ($2xLD_{50}$)

OXIMES	ED_{50} (mg/kg)	SR (LD_{50}/ED_{50})
Pralidoxime	10.45 (7.50 - 14.60)	22.1
Obidoxime	1.56 (1.07 - 2.28)	120.8
Methoxime	0.78 (0.61 - 1.00)	822.8
BI-6	1.97 (1.56 - 2.50)	135.2
HI-6	0.47 (0.38 - 0.57)	1428.3
HLö-7	0.16 (0.12 - 0.22)	2225.0

Table 4: Prophylactic antidotal potency (ED_{50}) and safety ratio (SR) in cyclosin poisoned mice ($2xLD_{50}$)

OXIMES	ED_{50} (mg/kg)	SR (LD_{50}/ED_{50})
Pralidoxime	52.99 (36.70 - 76.50)	4.4
Obidoxime	18.18 (11.20 - 29.50)	10.4
Methoxime	4.19 (2.41 - 7.29)	153.2
BI-6	0.51 (0.30 - 0.86)	522.2
HI-6	0.12 (0.08 - 0.20)	5594.2
HLö-7	0.45 (0.37 - 0.56)	791.1

Discussion

Our results clearly demonstrate large differences in the efficacy between conventional oximes and H oximes, especially against soman and cyclosin. The therapeutic efficacy of oximes depends on the type of nerve agent in spite of prophylactic administration of antidotes that reduces the nerve agent - specific influence of aging. It means that other aspects of nerve agent toxidynamics than aging can influence on the efficacy of oximes (2).

With conventional oximes, soman or cyclosin poisoned mice could only be effectively treated with high doses (usually more than 20% LD_{50}) but not with doses supposed for humans (approximately 2% LD_{50}), with the exception of methoxime against cyclosin. Only H oximes (especially HI-6 and HLö-7) were effective at „human“ doses in poisoned mice regardless of the nerve agent used (10). The new oxime BI-6 is sufficiently effective at „human“ dose in mice intoxicated with cyclosin but not with soman, where LD_{50} value corresponds to 6% LD_{50}.

The much higher therapeutic potency of H oximes in comparison with conventional oximes may be caused not only by the higher reactivating efficacy (6,9) but also by other antidotal mechanisms based on antimuscarinic, antinicotinic and ganglion blocking actions as well as on restoration of neuromuscular blockade and beneficial effects on cardiovascular and respiratory systems (4,8,12,18,19).

In conclusion, the present results indicate that only H oximes (HI-6, HLö-7) are effective against supralethal intoxication of mice when given in very low doses corresponding to those proposed for humans, regardless of the nerve

agent used. The new oxime BI-6, synthesized in our laboratory, is as effective as other H-oximes tested against sarin and cyclosin, but a little less efficacious against soman. Thus both H oximes (HI-6 and HLö-7) may be attractive compounds to develop for use against nerve agent poisoning in spite of their relative instability in aqueous solution and relative insolubility (7,14). They are being considered to replace the oximes used until now in military injectors although general decision to replace the currently used oximes by H oximes could not be reached (19).

Acknowledgements

The author expresses his appreciation to Mrs. J Petrová and Mrs. J. Uhlířová for their technical assistance and help with statistical evaluation. Special thanks are due to Dipl. ing. J. Bielavský for synthesis of the new oxime BI-6.

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Submitted January 1998.
Accepted January 1998.

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