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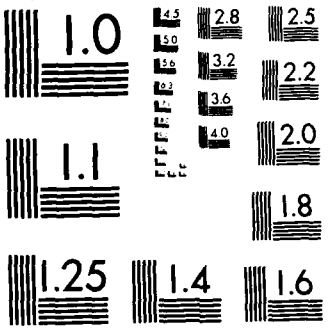

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OXIME-INDUCED REACTIVATION AND EFFICACY AGAINST LETHALITY IN PHOSPHINATE-INTOXICATED GUINEA PIGS

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Oxime-induced Reactivation and Efficacy Against Lethality in Phosphinate-intoxicated Guinea Pigs

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19 ABSTRACT (Continue on reverse if necessary and identify by block number)

To investigate the practicality of using organophosphinates (phosphinates) as pretreatments against nerve agent intoxication, experiments were conducted to determine whether oximes can induce reactivation of phosphinate-inhibited guinea pig acetylcholinesterase (AChE) and whether the toxicity of phosphinates is reduced by treatment with atropine and/or oxime. Three phosphinates, 4-nitrophenyl methyl(phenyl) phosphinate (MPP), 4-nitrophenyl chloromethyl(phenyl) phosphinate (CMPP), and 4-nitrophenyl 2-methoxyphenyl(methyl) phosphinate (MPMP), were used in these experiments. In the first group of experiments, 2-PAM or HI-6 was administered im 2 min after peak inhibition of whole blood AChE activity by MPP or CMPP in vivo. Both oximes significantly reactivated each inhibitor; however, HI-6 was the better reactivator in both cases. Four of the five oximes used to reactivate MPMP exacerbated inhibition, and the fifth, TMB-4, gave only slight reactivation. In vitro studies using five different oximes to reactivate MPP-inhibited guinea pig erythrocyte AChE showed that HI-6 ($1x10^{-4}$M) gave almost total reactivation (91% of control). Toxogonin, HI-6, and

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19. ABSTRACT. cont'd.

TMB-4 ($5x10^{-5}M$) gave similar reactivation (94%) of CMPP inhibited AChE. Oximes were comparably poor reactivators of MPMP-inhibited AChE, with HI-6 giving the best reactivation (21%). Efficacy studies revealed that neither HI-6 nor 2-PAM potentiated the toxic effects of MPP or CMPP and atropine/oxime therapy provided greater protection (20 LD50s protection) against either phosphinate than any single therapy. The reactivation and efficacy data, especially for CMPP, support the contention that phosphinates may be useful as pretreatments against nerve agent intoxication.

FOREWORD

This research was done under protocol 1-02-84-004-A-294, which is part of Research Plan 1-02-0-04-0000 (formerly 250-81-001), entitled "Efficacy of Centrally and Peripherally Active Pretreatment Compounds Against Nerve Agent Intoxication," under Project 3M1627-34-A, Task Area 875AA (formerly AC), Work Unit 215. Paula A. Dennis, Leslie R. Holzinger, Ronnie S. Maloy, Willard J. Lennox and Brian G. Talbot also participated. we would like to acknowledge the assistance of Drug Testing and Evaluation Branch personnel during the efficacy portion (Experiment 3) of these studies.

OBJECTIVE AND LOCATION OF DATA

Objective: These experiments were designed to determine whether oximes can induce reactivation of inhibited blood AChE in animals given organophosphinates (phosphinates). The questions addressed were (1) whether oximes can reactivate phosphinate-inhibited guinea pig AChE in vivo and in vitro, (2) which oxime and concentration is most effective in restoring AChE activity, (3) nature of the profiles of AChE inhibition by phosphinates in vivo, and (4) evaluation of the effectiveness of atropine + oximes against phosphinate-induced lethality.

Location of Data: The data are located in Notebooks 104-84, 65-85, and 13-86.

TABLE OF CONTENTS

	PAGE
LIST OF TABLES AND FIGURES	vii
INTRODUCTION	1
MATERIALS AND METHODS	2
RESULTS	5
CONCLUSIONS AND RECOMMENDATIONS	11
REFERENCES	13
DISTRIBUTION LIST	15

LIST OF TABLES AND FIGURES

TABLES PAGE

1 Efficacy of Various Therapies against MPP- or CMPP-induced Lethality..... 10

2 Reactivation of Phosphinate-inhibited Guinea Pig RBC AChE In Vitro..... 10

FIGURES

1 Structures of the Three Phosphinates used in These Experiments..... 2

2 Spontaneous Reactivation of Inhibited Guinea Pig Whole Blood Acetylcholinesterase In Vivo..... 5

3a In Vivo Reactivation of MPP-inhibited Guinea Pig Whole Blood AChE by 2-PAM..... 6

3b In Vivo Reactivation of CMPP-inhibited Guinea Pig Whole Blood AChE by 2-PAM..... 7

4a In Vivo Reactivation of MPP-inhibited Guinea Pig Whole Blood AChE by HI-6..... 8

4b In Vivo Reactivation of CMPP-inhibited Guinea Pig Whole Blood AChE by HI-6..... 8

4c In Vivo Reactivation of MPMP-inhibited Guinea Pig Whole Blood AChE by Five Oximes..... 9

5 In Vitro Oxime Reactivation of Phosphinate-inhibited Guinea Pig Erythrocyte Acetylcholinesterase..... 11

INTRODUCTION

Recent studies have shown that pretreatment with carbamates and cholinolytic(s) or with carbamates followed by atropine/oxime therapy is effective in reducing the lethality of a wide variety of organophosphorus (OP) anticholinesterase compounds, including soman [1-5]. This enhanced protection is attributed to the ability of carbamates to inhibit a fraction of the total pool of acetylcholinesterase (AChE) enzyme, thus protecting it from irreversible inhibition by soman or similar agents. Spontaneous decarbamylation of this inhibited fraction of enzyme is then thought to provide a sufficient amount of AChE activity for normal function at some time after the agent has been bound, hydrolyzed, or eliminated. However, the protection lasts for only a short time since the half-time for spontaneous decarbamylation of the inhibited AChE is between approximately 30 and 50 min, depending on the carbamate [6] and species [7]. If protection is desired for longer periods of time, protective inhibition must be prolonged. One way of accomplishing this would be to administer an irreversible OP AChE inhibitor (one that does not undergo aging) that could be subsequently displaced by oxime administration reactivating the enzyme.

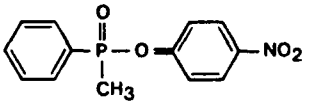
The use of OP pretreatment to protect against soman was first tested in 1971 by Berry et al. [8]. They were able to markedly reduce the toxicity of soman, i.e., increase the protective ratio, by prior administration of an OP (tetraethyl pyrophosphate, diethyl 4-nitrophenyl phosphate, ethyl 4-nitrophenyl methylphosphonate, etc.), followed by oxime and atropine. Further evidence to support the contention that the OP or carbamate protected vital AChE from irreversible inhibition by soman was provided in 1978 by Dirnhuber and Green [9]. They demonstrated that it was possible to reverse soman-depressed tetanus tension by reactivating the AChE previously inhibited by VX. Thus, any oxime-sensitive OP compound might be expected to afford protection against an oxime-resistant anticholinesterase compound (e.g., soman). It is unlikely that VX or TEPP would be used as a pretreatment for soman intoxication due to their toxicity. Phosphinates, however, are a class of AChE inhibitors in which some compounds are irreversible, do not age [10], and have toxicities similar to that of carbamates [11]. In vitro studies on the reactivation of phosphinate-inhibited eel AChE by oximes have been conducted in this laboratory, indicating phosphinate-inhibited AChE can be reactivated by oximes [12,13]. Also, some undergo spontaneous dephosphinylation. A candidate OP pretreatment compound must be responsive to oxime-induced reactivation of inhibited AChE and its own toxicity must be reduced by treatment with oxime and atropine. In this study we investigated whether these criteria are true for three candidate pretreatment phosphinates.

MATERIALS AND METHODS

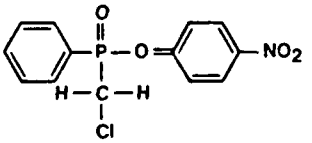
Animals: All experiments in this protocol were conducted using male guinea pigs [CRL:(HA)BR, 350-400 g].

Experiment 1: Profile of AChE-inhibition and spontaneous reactivation. Initially, a range-finding study of organophosphinates was conducted to establish dosages necessary to inhibit blood AChE activity by about 70%. Three phosphinates [4-nitrophenyl methyl(phenyl) phosphinate (MPP), 4-nitrophenyl chloromethyl(phenyl)phosphinate (CMPP), and 4-nitrophenyl 2-methoxyphenyl(methyl)phosphinate (MPMP)] were used in these experiments (Fig. 1). These compounds were synthesized for the Army by Ash Stevens Inc. under contract DAMD17-84-C-4235. Each compound was characterized by a satisfactory elemental analysis and an IR and NMR spectrum in consonance with the assigned structure. Vehicle for the phosphinates was 5% EtOH:95% PEG-200. The phosphinates were given intramuscularly (im). Blood samples were obtained by clipping the tip of the toenail and collecting the blood in a heparinized capillary tube. Using the radiometric method described by Siakotos et al. [14], blood was assayed for AChE activity before and at various times after administration of MPP, CMPP, MPMP.

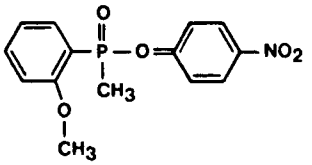
Figure 1.
STRUCTURES OF THE THREE PHOSPHINATES USED IN THESE EXPERIMENTS



4-NITROPHENYL-METHYL(PHENYL) PHOSPHINATE (MPP)



4-NITROPHENYL-CHLOROMETHYL(PHENYL) PHOSPHINATE (CMPP)



4-NITROPHENYL-2-METHOXYPHENYL(METHYL) PHOSPHINATE (MPMP)

Experiment 2: In vivo reactivation of phosphinate-inhibited AChE by oxime. Two oximes were originally chosen for reactivation of AChE: 2-(hydroxyimino)- methyl-1-methylpyridinium chloride (2-PAM) was chosen because it is the standard antidote in the treatment of nerve agent intoxication; 1-[(2-hydroxyimino)methylpyridinio]-3-(4-carbamoyl-pyridinio)-2-oxapropane dichloride (HI-6), because it is a novel oxime which is effective against soman intoxication. The dosages of oximes to be used in the MPP and CMPP experiments were those used previously by Green et al. [15] to establish a therapeutic range against sarin intoxication. Their data showed a linear relationship between 2-PAM dosage (i.e., 3.12, 6.25, 12.5 and 25.0 mg/kg, im) and efficacy against lethality. Dosages of HI-6 equimolar to the above 2-PAM dosages were also given. Reactivation of MPMP-inhibited AChE was done using five oximes (2-PAM, HI-6, TMB-4, Toxogonin, and HGG-12 at 145 umoles/kg). For each phosphinate, oxime was administered 2 min after maximum AChE inhibition was observed (60 min for MPP and CMPP; 120 min for MPMP). Oximes were dissolved in twice-distilled water. All im injections (to include oximes, inhibitors, and vehicles) were in a volume equivalent to 0.5 ml/kg body weight. In addition, a separate group of vehicle-treated animals was used. The details for this experiment are outlined below:

	Dosage of Oxime					
Inhibitor	umoles/kg,im	mg/kg 2-PAM	mg/kg HI-6	mg/kg TMB-4	mg/kg TOX	mg/kg HGG-12
MPP or CMPP	18.1	3.12	7.17	-	-	-
MPP or CMPP	36.1	6.25	14.3	-	-	-
MPP or CMPP	72.7	12.5	28.7	-	-	-
MPP or CMPP	145.3	25.0	57.4	-	-	-
MPMP	145.3	25.0	57.4	67.4	52.2	66.4
MPP, CMPP or MPMP	vehicle	-	-	-	-	-
vehicle	145.3	25.0	57.4	67.4	52.2	66.4
vehicle	vehicle	-	-	-	-	-

Blood was taken for measurement of AChE activity before inhibitor (control), 30, 45, 60, 75, 90, 120 and 180 min after MPP and CMPP, and 60, 90, 120, 135, 150, 165, 180, and 240 min after MPMP. Control AChE activity was used as the baseline from which the oxime-induced reactivation was estimated.

Experiment 3: Efficacy against phosphinate-induced lethality. The following experiments were conducted to test the effectiveness of oxime therapy, alone and together with atropine, 16 mg/kg, against MPP- and CMPP-induced lethality.

The 145 uM/kg dose of each oxime was tested against each inhibitor. For seven levels of inhibitor, two animals for each treatment were injected at 0.15 log intervals to bracket the 24 hr LD50 values for each inhibitor/treatment combination as a range-finding study.

The details for this experiment are outlined below:

Lethality Study

Inhibitor	Treatment[1]
MPP or CMPP	vehicle
MPP or CMPP	atropine
MPP or CMPP	atropine + HI-6
MPP or CMPP	HI-6
MPP or CMPP	atropine + 2-PAM
MPP or CMPP	2-PAM

[1] Treatment, im, given in the opposite leg 1 min after inhibitor, im.

Using the treatments outlined above, protective ratios were then determined in male guinea pigs using the treatments outlined above; each treatment was comprised of 5 doses of inhibitor and 6 animals/dose. For each inhibitor, all treatment groups were run in parallel in a randomized block fashion.

Experiment 4: Effects of oximes on phosphinate-inhibited guinea pig erythrocyte AChE *in vitro*. Anticoagulant-treated guinea pig whole blood (4 ml) was incubated for 30 min with vehicle or inhibitor (MPP, CMPP, or MPMP) to obtain in excess of 70% erythrocyte AChE inhibition. Erythrocytes were then washed four times (10 ml/wash) with cold saline to remove excess inhibitor. Aliquots (0.2 ml) of packed erythrocytes were added to 0.6 ml of phosphate buffer preparation (final incubation conditions: phenylmethylsulfonylfluoride, 7.5 ug; NaCl, 75 mM; CaCl2, 0.75 mM; MgCl2, 1.5 mM; phosphate buffer, 37.5 mM, pH 7.3) alone or with oxime. These mixtures were incubated for 30 min, and washed three times with cold saline; aliquots of the packed erythrocytes were assayed for AChE activity using the radiometric method of Siakotos *et al*. An outline of the *in vitro* studies run with the respective phosphinates is given below:

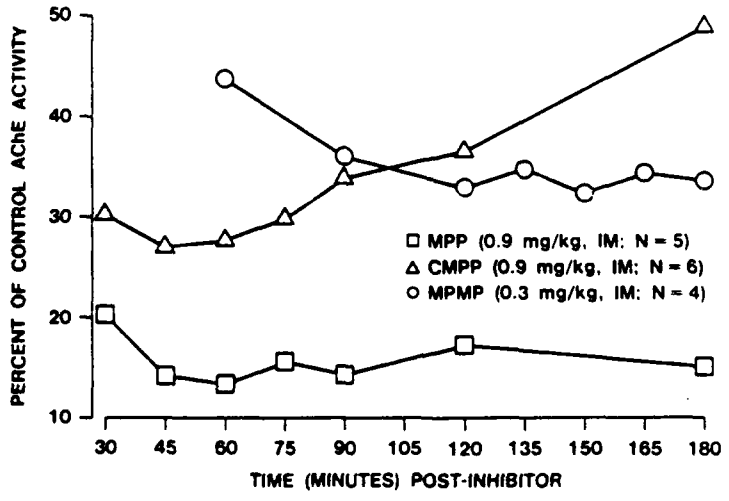
Phosphinate (Concentration)	Oxime Concentration	N[1]	Oxime Used: 2-PAM	HI-6	HGG-12	TMB-4	TOX
MPP	$1x10^{-4}$M		+	+	+	+	+
($5x10^{-6}$M)	$2.5x10^{-6}$ to $2.5x10^{-4}$M	8		+			
CMPP	$5x10^{-5}$M		+	+	+	+	+
($4.4x10^{-6}$M)	$1x10^{-6}$ to $1x10^{-4}$M	8		+			
	$2.5x10^{-5}$ to $2.5x10^{-3}$M	7	+				
MPMP	$2x10^{-3}$M		+	+	+	+	+
($3.3x10^{-6}$M)	$2x10^{-4}$ to $1.6x10^{-2}$M	7		+			

[1] Number of concentrations within the range specified; includes control.

RESULTS

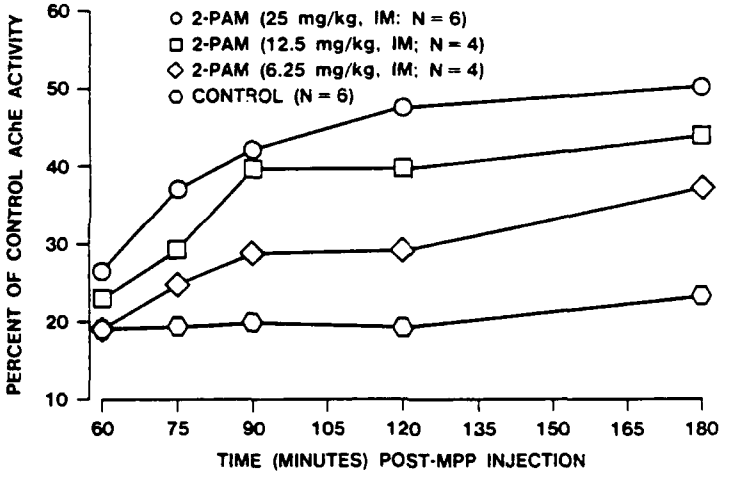
Experiment 1. Profiles of AChE inhibition and spontaneous reactivation for MPP, CMPP, and MPMP are illustrated in Fig. 2. CMPP was the only phosphinate tested that showed significant spontaneous reactivation over the sampling period. Maximum AChE inhibition was measured at 60 min for MPP and CMPP, and at 120 min for MPMP.

Figure 2.
SPONTANEOUS REACTIVATION OF INHIBITED GUINEA PIG WHOLE BLOOD ACETYLCHOLINESTERASE IN VIVO



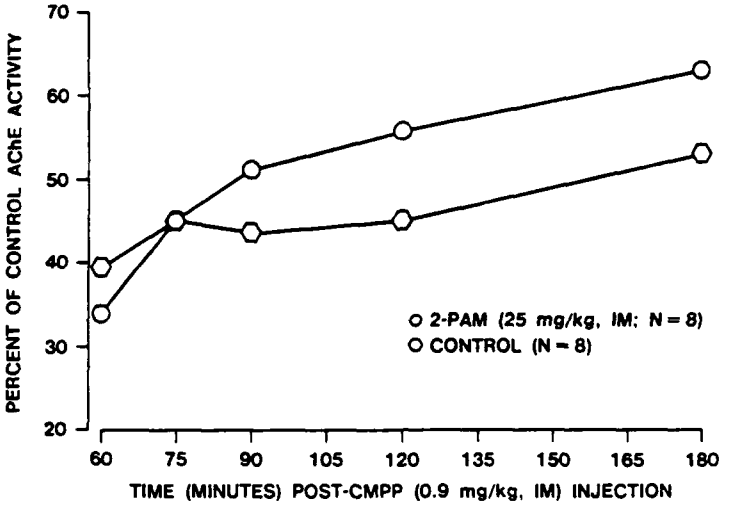
Experiment 2. In the MPP and CMPP experiments, guinea pigs were injected with MPP or CMPP, and 62 min after phosphinate (at maximum AChE inhibition), oxime was administered to assess its effect on the inhibited AChE. Figs. 3a and 3b illustrate the effects of 2-PAM on AChE activity of MPP- and CMPP-treated guinea pigs respectively. For MPP, 12.5 mg/kg 2-PAM was the lowest concentration which significantly ($p<.05$) increased AChE activity above control at all time points. However, AChE activity at 6.25 mg/kg of 2-PAM differed from control ($p<.05$)

Figure 3a.
IN VIVO REACTIVATION OF
MPP-INHIBITED GUINEA PIG WHOLE BLOOD AChE
BY 2-PAM



starting at 90 min. For CMPP, it can be seen in Fig. 3b that 2-PAM (25 mg/kg) has significantly shifted the curve upward so that from 60 to 90 min it is not parallel to the CMPP control curve, thus indicating induced reactivation. Also, significant CMPP control group ($p<.05$) spontaneous reactivation occurred from 60 to 180 min and this group generally approached control AChE activity levels by 24 hrs.

Figure 3b.
IN VIVO REACTIVATION OF
CMPP-INHIBITED GUINEA PIG WHOLE BLOOD AChE
BY 2-PAM



The effects of HI-6 on MPP- and CMPP-inhibited guinea pig AChE activity in vivo are shown in Figs. 4a and 4b. All doses of HI-6 significantly ($p<.05$) enhanced MPP-inhibited AChE activity. The degree of reactivation effected by these four doses of HI-6 were not statistically different. Similar results were achieved for CMPP-inhibited AChE. Significant spontaneous reactivation ($p<.05$) was observed in the CMPP control group from 60 to 180 min (Fig. 4b). HI-6 was more effective at reactivating phosphinate-inhibited AChE at lower doses and more rapidly than equimolar doses of 2-PAM. Overall, the highest oxime dose (145 uM) gave the greatest reactivation of inhibited AChE and was therefore chosen for use in the lethality studies as well as in in vivo experiments involving MPMP.

In the MPMP experiments, guinea pigs were injected with vehicle or MPMP; then 2 min after peak AChE inhibition (inhibition peaked at 120 min), oxime (145 uM/kg, im) or vehicle was injected to effect a change in AChE activity. There was no significant difference among the six treatments (Fig. 4c); however, when the TMB-4 group was excluded from the ANOVA, a significant difference was detected ($p=.019$) among treatments. This was due to the small sample size ($N=2$) and large standard deviations around the mean of the replicates in the TMB-4-treated animals. Unlike the observations with MPP and CMPP, the oximes tended to exacerbate MPMP inhibition of AChE. For this reason, lethality studies involving MPMP were omitted.

Figure 4a.
IN VIVO REACTIVATION OF
MPP-INHIBITED GUINEA PIG WHOLE BLOOD AChE
BY HI-6

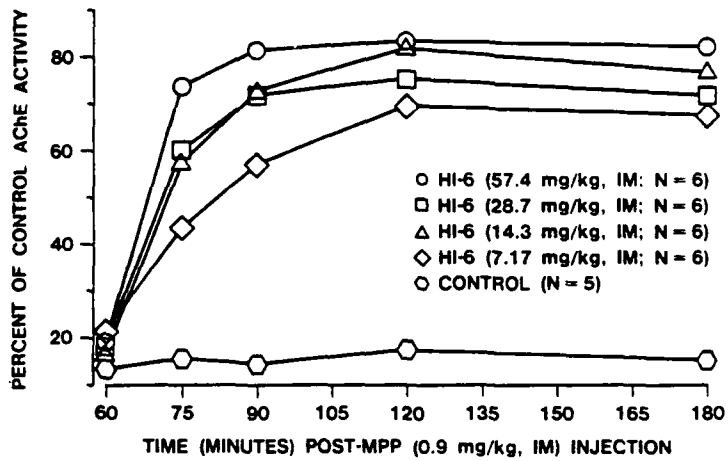


Figure 4b.
IN VIVO REACTIVATION OF
CMPP-INHIBITED GUINEA PIG WHOLE BLOOD AChE
BY HI-6

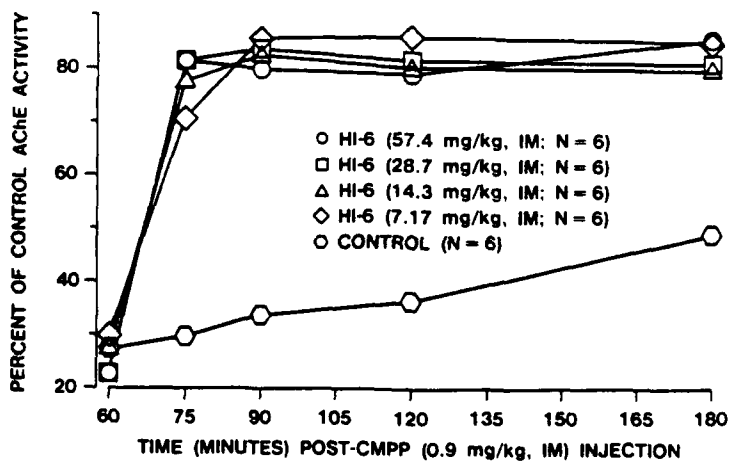
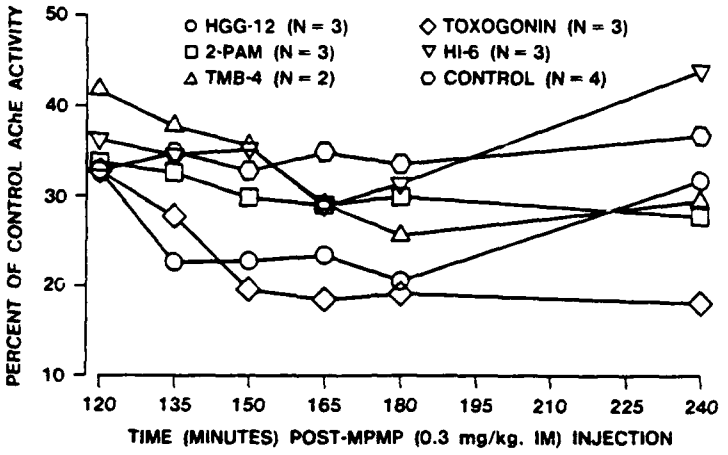


Figure 4c.
IN VIVO REACTIVATION OF
MPMP-INHIBITED GUINEA PIG WHOLE BLOOD AChE
BY FIVE OXIMES



Experiment 3. Results of efficacy testing of atropine and/or oxime therapy against MPP- and CMPP-induced lethality are shown in Table 1. Data were analyzed using parallel lines probit analysis [15] except where noted. Problems encountered during the CMPP lethality study resulted in quite variant data; therefore, a second CMPP lethality study was run. Since deaths occurred after 24 hrs, a 48-hr LD50 and protective ratios (Table 1) were calculated for the CMPP study. Problems with the two CMPP lethality studies may have been due to 1) the higher doses of our injection solutions coming out of solution as the study progressed and having to be resonicated, and 2) large injection volumes (up to 1.41 ml/kg, im) having been absorbed at a different rate than the 0.5 ml/kg, im injections that most animals received. These problems probably led to inconsistent injections. Regardless of how variant the CMPP data may appear, it is important to note that neither oxime potentiated the toxic effects of CMPP, and that atropine/oxime therapy provided marked protection against this phosphinate.

Experiment 4. The data in Table 2 illustrate the effects of oximes on phosphinate-inhibited guinea pig erythrocyte AChE <u>in vitro</u>. The order of reactivation for the five oximes (oxime concentration) against each phosphinate was MPP (1 X 10-4M): HI-6 > Toxogonin = TMB-4 > HGG-12 = 2-PAM > Control; CMPP (5 X 10-5M): Toxogonin = HI-6 = TMB-4 > HGG-12 > 2-PAM = Control; MPMP (2 X 10-3M): HI-6 = Toxogonin = TMB-4 > HGG-12 = 2-PAM = Control. HI-6, Toxogonin, and TMB-4 are the best reactivators of the three phosphinates. With MPMP, little AChE reactivation occurred as compared to the other two phosphinates and a much higher oxime concentration was used against this inhibitor.

Table 1. Efficacy of Various Therapies against MPP- or CMPP-induced Lethality.

	Anticholinesterase Compound							
	CMPP				MPP			
Therapy[1]	LD50[2]	(95% CL)	PR[3]	(95% CL)	LD50	(95% CL)	PR	(95% CL)
Control	6.28	(2.99-10.4)	1.0	NA	2.36	(1.76-3.11)	1.0	NA
	6.22	(3.64-9.38)[4]	1.0	NA				
2-PAM	13.5	(8.26-23.1)	2.15	(1.09-5.64)	5.27	(3.98-6.97)	2.23	(1.5-3.34)
	13.2	(9.06-19.6)	2.12	(1.23-4.22)				
HI-6	68.2	(38.8-111.)	10.9	(5.36-25.7)	14.5	(10.5-19.2)	6.13	(4.0-9.11)
	64.9	(42.6-94.3)	10.4	(5.95-19.8)				
Atropine	312.	(177.-785.)	49.7	(22.8-195.)	5.99	(4.28-8.07)	2.54	(1.65-3.8)
	164.	(113.-252.)	26.4	(15.2-54.9)				
Atropine+ 2-PAM	542.	(334.-1050)	86.3	(42.9-261.)	127.	(94.8-179.)	54.	(36.2-85.3)
	208.	(129.-307.)	33.5	(18.9-62.7)				
Atropine+ HI-6	643.	(389.-1350)	102.	(49.9-337.)	218.	(158.-319.)	92.5	(60.6-150)
	148.	(80.1-231.)	23.7	(12.1-45.1)				

[1] Therapy, im given 1 min post-inhibitor, and consisted of 2-PAM (25 mg/kg), HI-6 (57.4 mg/kg), atropine (16 mg/kg), or a combination of oxime and atropine.

[2] LD50 (mg/kg, im).

[3] PR = Protective Ratio = (LD50 treated)/(LD50 control)

[4] 48 hr LD50, PR and CL values.

Table 2. Reactivation of Phosphinate-inhibited Guinea Pig RBC AChE In Vitro.

	Phosphinate (Concentration; sample size)					
	MPP ($5x10^{-6}$M; N=5)[1]		CMPP ($4.4x10^{-6}$M; N=6)[2]		MPMP ($3.3x10^{-6}$M; N=4)[3]	
Oxime	Mean Percent of Control AChE Activity	(S.D.)	Mean Percent of Control AChE Activity	(S.D.)	Mean Percent of Control AChE Activity	(S.D.)
None	9.33	(6.87)	30.6	(11.7)	2.67	(2.44)
2-PAM	47.4	(5.50)	44.0	(10.6)	6.36	(1.63)
HGG-12	52.7	(6.18)	77.3	(7.08)	8.58	(7.54)
TMB-4	71.7	(11.6)	94.7	(8.57)	15.0	(5.19)
Toxogonin	77.4	(11.1)	98.5	(8.32)	17.6	(10.3)
HI-6	90.9	(9.48)	97.9	(3.38)	21.1	(4.47)

[1] Oxime concentration = $1x10^{-4}$M; Order of oxime effectiveness: HI-6 > Toxogonin = TMB-4 > HGG-12 = 2-PAM > control.

[2] Oxime concentration = $5x10^{-5}$M; Order of oxime effectiveness: Toxogonin = HI-6 = TMB-4 > HGG-12 > 2-PAM = control.

[3] Oxime concentration = $2x10^{-3}$M; Order of oxime effectiveness: HI-6 = Toxogonin = TMB-4 > HGG-12 = 2-PAM = control.

Figure 5.
IN VITRO OXIME REACTIVATION OF
PHOSPHINATE-INHIBITED
GUINEA PIG ERYTHROCYTE ACETYLCHOLINESTERASE

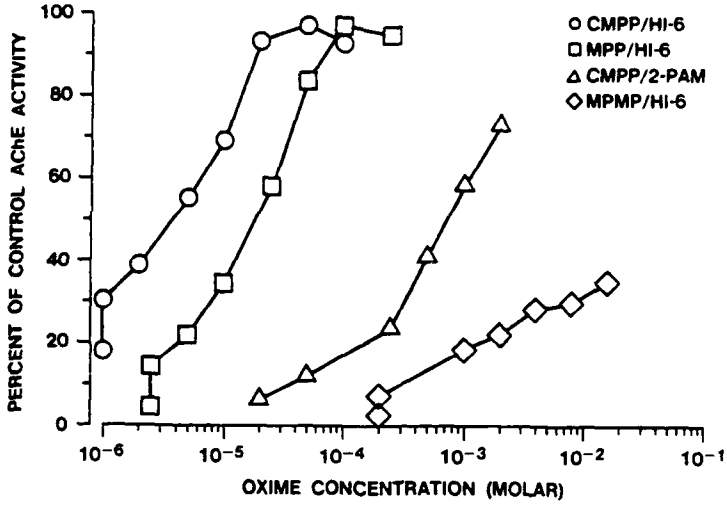


Fig. 5 illustrates the effects of various concentrations of 2-PAM or HI-6 on phosphinate-inhibited erythrocyte AChE. It is apparent in Fig. 5 that CMPP-inhibited AChE is more readily reactivated by lower concentrations of HI-6 than is AChE inhibited by the other two phosphinates. It can also be seen in Fig. 5 that HI-6 reactivates CMPP-inhibited AChE at lower concentrations than that required by 2-PAM.

CONCLUSIONS AND RECOMMENDATIONS

1. HI-6 and 2-PAM therapies significantly reactivated the inhibited AChE activity in MPP- or CMPP-treated guinea pigs. Conversely, none of the 5 oximes tested against MPMP-treated guinea pigs reactivated the inhibited AChE; rather, they tended to exacerbate the AChE inhibiting effects of MPMP.

2. Neither HI-6 nor 2-PAM potentiated the toxic effects of MPP or CMPP, and atropine/oxime therapy provided greater protection against either phosphinate.

3. HI-6, Toxogonin, and TMB-4 significantly enhanced MPP- and CMPP-inhibited AChE activity, whereas the oximes had little effect on MPMP-inhibited erythrocytes.

4. The reactivation and efficacy data support the supposition that phosphinates should be useful as pretreatments against nerve agent intoxication. It is recommended that CMPP pretreatment in conjunction with atropine plus oxime be tested against soman to establish whether this regimen is superior to carbamate pretreatment and atropine plus oxime therapy.

REFERENCES

1. Fleisher, J. H. and Harris, L. W. (1965). Dealkylation as a mechanism for aging of cholinesterase after poisoning with pinacolyl methylphosphonofluoridate. Biochem. Pharmacol., 14, 641-650.

2. Berry, W. K. and Davies, D. R. (1970). The use of carbamates and atropine in the protection of animals against poisoning by 1,2,2-trimethylpropyl methylphosphonofluoridate. Biochem. Pharmacol., 19, 927-934.

3. Dirnhuber, P., French, M. C., Green, D. M., Leadbeater, L., and Stratton, J. A. (1979). The protection of primates against soman poisoning by pretreatment with pyridostigmine. J. Pharm. Pharmacol., 31, 295-299.

4. Gordon, J. J., Leadbeater, L. and Maidment, M. P. (1978). The protection of animals against organophosphate poisoning by pretreatment with a carbamate. Toxicol. Appl. Pharmacol., 43, 207-216.

5. Heyl, W. C., Harris, L. W., and Stitcher, D. L. (1978). Effects of carbamates on blood cholinesterase activity: Chemical protection against soman. Drug Chem. Toxicol., 3, 319-332.

6. Reiner, E. (1971). Spontaneous reactivation of phosphorylated and carbamylated cholinesterase. Bull. Wld. Hlth. Org., 44, 109.

7. Harris, L. W., Talbot, B. G., Anderson, D. R., Lennox, W. J., Green, M. D. (1985). Apparent relationship between decarbamylation half-time and efficacy against soman lethality in different species. The Pharmacologist, 27, 140.

8. Berry, W. K., Davies, D. R. and Gordon, J. J. (1971). Protection of animals against soman by pretreatment with some other organophosphorus compounds, followed by oxime and atropine. Biochem. Pharmacol., 20, 125-134.

9. Dirnhuber, P. and Green, D. M. (1978). Effectiveness of pyridostigmine in reversing neuromuscular blockade produced by soman. J. Pharm. Pharmac., 30, 419-425.

10. Lieske, C. N., Clark, J. H., Meyer, H. G., Lowe, J. R., Lawson, M. A., Lennox, W. J., Sultan, W. E., Kaminskis, A., Groff, W. A., Shutz, M. B., and Singer, A. (1981). The concept and chemistry of organophosphinates as prophylactic agents in organophosphorus intoxication. 181st Nat. Mtg. Am. Chem. Soc., Atlanta, GA, Abstract MEDI 71.

11. Lieske, C. N. (1984). Unpublished results.

12. Harris, L. W., Stitcher, D. L., Heyl, W. C., Lieske, C. N., Lowe, J. R., Clark, J. H., and Broomfield, C. A. (1979). The effects of atropine-oxime therapy on cholinesterase activity and survival of animals intoxicated with p-nitrophenyl di-n-butylphosphinate. Toxicol. Appl. Pharmacol., 49, 23-29.

13. Anderson, D., Harris, L., Mireles, J., Lennox, W., Lieske, C., and Green, M. (1985). Induced reactivation by oxime of phosphinate-inhibited acetylcholinesterase in vitro and in vivo. Fed Proc., 44, 511.

14. Siakotos, A. N., Filbert, M. and Hester, R. (1970). A specific radioisotopic assay for acetylcholinesterase and pseudocholinesterase in brain and plasma. Biochem. Med., 3, 1-5.

15. Green, M. D., Jones, D., and Hilmas, D. (1984). Guinea pig model for correlating efficacy with 2-PAM plasma levels. USMRDC Fourth Ann. Chem. Def. Bioscience Review.

16. Finney, D. J. (1971). Probit Analysis, A Statistical Treatment of the Sigmoid Response Curve. Ind. Ed. Cambridge Univ. Press, Cambridge, England.

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