RESEARCH PAPERS

OXIME THERAPY IN POISONING BY SIX ORGANOPHOSPHORUS INSECTICIDES IN THE RAT

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The effects of two oxime reactivators, PAM and TMB4, on poisoning by six organophosphate indirect cholinesterase inhibitors with different phosphorylating groups have been examined in the rat. Repeated oxime injections were beneficial in poisoning by parathion, parathion-methyl, phenkaptone and, to a lesser extent, diazinon, but ineffective with dimefox and dimethoate. PAM had no additional antidotal value against dimethoate poisoning when given with atropine. The findings are related to expected degrees of irreversibility of cholinesterase inhibition with different phosphorus compounds.

IN recent years, considerable attention has been devoted to the treatment of organophosphate poisoning by specific cholinesterase reactivators, and a number of oximes have been found effective, particularly in conjunction with atropine. Until recently, attention was concentrated on pyridine-2-aldoxime methiodide (PAM), and work on this has recently been reviewed[1]. More recently, a number of other materials have been examined, and a further one, bis(pyridine-4-aldoxime) trimethylene dibromide (TMB4), found effective[1-9]. The greater effectiveness of TMB4 over PAM showed species variation[4,6,9]; TMB4 had the advantage of greater water solubility.

The mode of action of these oximes consists mainly of reactivation of inhibited cholinesterase by dephosphorylation[1,2,7-17], though there could occasionally be a slight additional benefit by direct reaction with the organophosphorus compound before inhibition occurs[1,11,13]. When used in combination with atropine, the benefit obtained was often far greater than expected from the individual effects of the materials alone[1,3,4,6,8-10,12,13,16,19]. Oxime reactivation of whole brain cholinesterase appears to take place *in vitro* as readily as that of other tissues, but *in vivo* the reactivation was relatively much slower or absent, suggesting that the oximes (for example, PAM and TMB4), possibly due to their ionic nature, would only slowly penetrate the blood-brain barrier to the nerve cells, though this may not necessarily apply to individual vital centres in the brain[1,9,15,16,19].

The reactivating efficiency of oximes varies with the nature of the enzyme-phosphorylating portion of the organophorphorus compound[20,21]; reactivation occurs most readily with diethyl phosphates such as TEPP and paraoxon, less readily with di-isopropyl phosphates such as DFP, and only to a negligible extent with the phosphoramide, schradan[1,8,9,13-16,18,22] No reactivation occurs *in vitro* or *in vivo* with the dimethyl phosphates

demeton-methyl or endothion such as occurs with parathion; there is some reduction in mortality with endothion, but less with demeton-methyl[23]. Work on parathion[1,17,23–25] suggests that with this diethyl indirect inhibitor, from which the anticholinesterase oxidation product is formed only slowly in the liver, repeated doses of oxime are required to maintain the cholinesterase reactivation *in vivo*; this is consistent with the finding that the blood concentration of PAM injected into rats falls to an ineffective level after 1–2 hours[13,16]. *In vitro* tests have shown that oxime reactivation of inhibited cholinesterase only occurs when the enzyme is at the reversibly-phosphorylated stage, and not after the phosphorylation has become irreversible[1,16,26,27]. This change from reversibly- to irreversibly-phosphorylated enzyme takes place much more readily with phosphoramide or with dimethyl or diisopropyl phosphate inhibition than with diethyl phosphate inhibition[1,13,16,26–28], and a greater proportion of irreversible inhibition occurs if the direct inhibitor is released or formed slowly, or is relatively persistent in the body[28].

Most reported work on oxime reactivation has thus been with direct inhibitors of cholinesterase, while dimethyl phosphate inhibitors have similarly received little attention. The necessity for repeated injections against parathion poisoning seems likely to apply to most indirect inhibitors, where the active metabolite is formed comparatively slowly. On the evidence of comparative rates of change to irreversible phosphorylation, the oxime reactivation rate after dimethyl phosphate poisoning might be more comparable with those found with diisopropyl than diethyl phosphates, or may approach zero as suggested for demeton-methyl and endothion[23], and by reversibility data[28].

It thus seemed of interest and importance to explore the therapeutic activity of the two oximes at present of greatest interest, PAM and TMB4, against a series of common indirect inhibitors of various types, including dimethyl phosphates, to determine whether the inclusion of oxime reactivators in therapy of poisoning by these compounds should be recommended.

Experimental Methods

PAM was obtained from Messrs. L. Light & Company, and TMB4 kindly supplied by Dr. F. Hobbiger; both were administered as 5 per cent w/v aqueous solutions. Atropine sulphate was BP grade administered as a 3·48 per cent w/v aqueous solution. Dimethoate (*00*-dimethyl-*N*-methylcarbamoylmethyl phosphorodithioate) was used as a high purity sample prepared in these laboratories, or as impure technical material, administered as a 40 per cent w/v propylene glycol solution. Diazinon and phenkaptone were pure samples obtained from J. R. Geigy S.A. (Basle), administered undiluted. Dimefox was a pure sample prepared in these laboratories, administered as a 0·4 per cent w/v propylene glycol solution. Parathion was 98·5 per cent technical material, administered as a 0·4 per cent w/v solution in glycerol formal[29]. Parathion-methyl was a pure sample supplied by Dr. W. N. Aldridge, administered as a 3·2 per cent w/v solution in glycerol formal.

Rats were semi-adult (150–200 g.) animals of Wistar strain, maintained and fed under standard conditions. Female rats were used in most experiments (exceptions being referred to in the text). Administration techniques were orthodox, and, except where killing early, by decapitation, for tissue cholinesterase assay was required, the observation period was seven days.

In certain experiments, plasma, erythrocyte and whole brain cholinesterase activities were determined by a standard manometric method[30], and the results expressed as percentages of a series of normal values obtained in this laboratory on similar rats.

In all experiments, the organophosphate compound was given orally, with intraperitoneal injections of the oxime or atropine 15 minutes before and 4 hours after this, followed by a further subcutaneous oxime or atropine injection after 8 hours, as a "maintenance dose". Each dose of PAM was 100 mg./kg., and each dose of TMB4 25 mg./kg., these being the highest tolerated without toxic effect. Preliminary tests suggested a single dose intraperitoneal LD50 of TMB4 to the female rat of about 80 mg./kg., and of PAM about 400 mg./kg.; effects consisted of dyspnoea, convulsions and respiratory failure, as previously reported[8,9,31]. The dose of atropine sulphate used was 17·4 mg./kg.

Symptom intensities were assessed on a numerical basis, as follows. 1. Slight fibrillation. 2. Moderate fibrillation. 3. Severe toxic effects, animal mobile. 4. Severe toxic effects, animal prostrate.

Results

Approximate oral LD50 values to female rats were first obtained on the organophosphates, as follows.

Dimethoate, pure	600 mg./kg.
Dimethoate, technical ..	120 mg./kg. (male rats)
Parathion	3 mg./kg. (5 mg./kg. to male rats)
Parathion-methyl ..	16 mg./kg. (12 mg./kg. to male rats)
Diazinon	800 mg./kg.
Phenkaptone	50 mg./kg.
Dimefox	1·8 mg./kg.

The low toxicity of the diazinon and its very slow onset of effects, were noteworthy, and believed due to its unusually high purity[32].

Each of the organophosphates was then given orally, in doses slightly greater than the approximate LD50, to three groups of ten female rats. One of these groups served as control, the second received three injections of PAM, and the third three injections of TMB4, as detailed above. Mortalities and severity of effects were then recorded for seven days, and are summarised in Table I. There was initial narcosis with dimethoate, possibly less severe in the oxime-treated groups, and with phenkaptone in the groups receiving oxime only.

This experiment was then repeated using groups of six female rats and an organophosphate dose half the approximate LD50. Survivors were killed after 24 hours for plasma, erythrocyte and whole brain

cholinesterase assay. The results are summarised in Table II. A further similar experiment with a 50-hour observation period is summarised in Table III.

TABLE I

EFFECT OF OXIME THERAPY ON LETHAL ORGANOPHOSPHATE POISONING IN FEMALE RATS

Organophosphate and oral dosage	Therapy xl	Severity of effects*							Mortality (10 in group)						
		½ h	1 h	2 h	8 h	1 d	2 d	4 d	½h	1h	4h	8h	1d	2d	7d
Dimethoate (pure) (750 mg./kg.)	Nil	0	1	3–4	4	4	—	—	0	0	2	4	9	10	10
	PAM	0	0	3–4	4	4	—	—	0	0	0	0	5	10	10
	TMB4	0	0	3	4	4	—	—	0	0	0	1	1	10	10
Parathion (4 mg./kg.)	Nil	1–2	3–4	3–4	4	2	1–2	0	0	4	5	6	6	6	7
	PAM	0	1	1	1–2	0–1	0	0	0	0	0	0	0	1	1
	TMB4	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Parathion-methyl (20 mg./kg.)	Nil	3–4	4	3	1	1	0	0	4	9	9	9	9	9	9
	PAM	3–4	3–4	3	2	2–3	2–3	0	0	0	0	0	0	0	0
	TMB4	3–4	3–4	3–4	2	2–3	2–3	0–2	0	0	0	0	0	0	1
Diazinon (1000 mg./kg.)	Nil	0	0	0	1–2	3	4	0	0	0	0	0	0	4	9
	PAM	0	0	0	1–2	3	3–4	0–1	0	0	0	0	0	1	3
	TMB4	0	0	0	2–3	3–4	3	0	0	0	0	0	0	2	3
Phenkaptone (60 mg./kg.)	Nil	0	0	1–2	3–4	2–4	1–4	0	0	0	2	3	3	5	5
	PAM	0	0	0–2	2–4	1–2	1–2	0	0	0	0	0	0	0	0
	TMB4	0	0	1–3	3–4	1–2	0–1	0	1	1	1	1	1	1	1
Dimefox (2·2 mg./kg.)	Nil	0	0	0	3–4	2–3	1–2	0	0	0	0	4	7	7	8
	PAM	0	0	0	2–4	2–3	1–2	0	0	0	0	3	6	6	6
	TMB4	0	0	0	1–4	1–3	1–2	0–1	0	0	0	2	5	5	5

xl PAM dose 100 mg./kg. TMB4 25 mg./kg. Administered i.p. at 15 min. before and 4 hr. after, and s.c. at 8 hr. after organophosphate.

Code for toxic effects:
* 1. Slight fibrillation
2. Moderate fibrillation
3. Severe toxic effects, but animal mobile
4. Severe toxic effects, animal prostrate

TABLE II

EFFECT OF OXIME THERAPY ON SUB-LETHAL ORGANOPHOSPHATE POISONING AND CHOLINESTERASE INHIBITION IN FEMALE RATS

Organophosphate and oral dosage	Therapy	Severity of effects*						ChE at 24 hrs. Per cent normal		
		½ h	1 h	2 h	4 h	8 h	24 h	Erythrocyte	Plasma	Brain
Dimethoate (pure) (300 mg./kg.)	Nil	0	1	3	3–4	3	0–1	16	23	26
	PAM	0	0	2	3	3	2	20	19	20
	TMB4	0	0	2	3–4	3–4	1–3	15	28	24
Parathion (1·5 mg./kg.)	Nil	0	1–2	2	2	0–1	0	55	81	102
	PAM	0	0	0	0	0	0	73	83	107
	TMB4	0	0	0	0	0	0	78	95	96
Parathion-methyl (8 mg./kg.)	Nil	3–4	4	2–3	1–2	0–1	0	30	41	50
	PAM	2–4	2–4	2–3	1–2	0–2	0–1	34	41	42
	TMB4	1–3	2–4	2	1–2	0–2	0–1	23	28	43
Diazinon (400 mg./kg.)	Nil	0	0	0	0	0–1	2	9	5	18
	PAM	0	0	0	0	0–1	2–3	14	9	17
	TMB4	0	0	0	0	0–1	1–2	43	24	24
Phenkaptone (25 mg./kg.)	Nil	0	0	0	2–3	2–4	0–1	22	46	42
	PAM	0	0	0	1–2	1–2	0–1	61	58	37
	TMB4	0	0	0	1–2	2–3	0–1	50	40	40
Dimefox (0·9 mg./kg.)	Nil	0	0	0	0–1	1–2	0–2	26	33	98
	PAM	0	0	0	0–1	1–2	0–2	26	29	92
	TMB4	0	0	0	0	1–2	1–2	29	37	95

Code for toxic effects:
* 1. Slight fibrillation
2. Moderate fibrillation
3. Severe toxic effects, but animal mobile
4. Severe toxic effects, animal prostrate

Mortality at 24 hrs:—Parathion-methyl (untreated) 2/6
Phenkaptone (untreated) 1/6
Dimefox (treated TMB4) 1/6

TABLE III

EFFECT OF PAM THERAPY ON ORGANOPHOSPHATE POISONING AND CHOLINESTERASE INHIBITION IN MALE RATS

Organophosphate	Dose mg./kg.	Therapy	Effects of PAM on symptoms	Mortality 50 hour	ChE at 50 hours (per cent normal)		
					Erythrocyte	Plasma	Brain
Dimethoate (technical)	100	Nil PAM	None detectable	1/6 1/6	23 26	42 25	28 27
Parathion-methyl	12	Nil PAM	Slower recovery	3/6 0/6	37 38	49 54	47 28
Parathion	3	Nil PAM	Obviously less severe; faster recovery	0/6 1/6	36 56	58 90	86 89

A series of experiments was then performed to assess the effect of PAM on atropine therapy of dimethoate poisoning. Groups of ten male rats were given oral doses of pure or technical dimethoate, certain groups receiving three injections of atropine sulphate, or PAM, or both. Mortalities obtained are summarised in Table IV. PAM alone had little apparent

TABLE IV

EFFECT OF PAM AND ATROPINE THERAPY ON MORTALITY FROM ORAL DIMETHOATE POISONING IN FEMALE RATS

Dimethoate dose (mg./kg.) and grade	Therapy			
	Nil	PAM (100 mg./kg. × 3)	Atropine (17·4 mg./kg. × 3)	PAM + atropine
150 tech.	8/10	—	0/10	3/10
600 pure	5/10	8/10	0/10	3/10*
600 pure	5/10	6/10	0/10	2/10

* One of these deaths possibly not attributable to chemicals used.

effect; atropine gave slower development of effects and death, with almost complete control of secretions for 12 hours and considerably less weakness; atropine and PAM together gave an improvement similar to that with atropine alone, except that control of urinary incontinence appeared a little less lasting or effective.

In one test (see Table IV), some toxic effects were noted due to administration of PAM and atropine sulphate together; this anomalous result may have arisen from the use of an aged batch of PAM for this test, and did not occur when the test was repeated with newer material. Simultaneous administration of 17·4 mg./kg. atropine intraperitoneally reduced the intraperitoneal LD50 of PAM from about 400 to about 150 mg./kg.

DISCUSSION

Examination of Tables I–IV showed that oxime therapy alone was of no apparent value in poisoning by dimethoate or dimefox. With diazinon there was some reduction of mortality, together with reduced erythrocyte and plasma cholinesterase inhibition particularly with TMB4. With phenkaptone there was reduction in mortality and (particularly with PAM) of symptom severity, with reduced erythrocyte cholinesterase

inhibition. The narcosis occurring with oxime therapy of phenkaptone poisoning cannot at present be explained. With parathion there was marked reduction of mortality and symptom severity, accompanied by reduced erythrocyte and plasma cholinesterase inhibition. With parathon-methyl there was marked reduction in mortality but not in symptom severity, recovery being delayed, with no apparent reduction in cholinesterase inhibition.

These results are largely consistent with observations on reversibility of inhibition previously reported[13,16,26–28]. Dimefox is a phosphoramide similar to schradan, whose inhibition appears almost entirely irreversible[1,13,24,25], so that the lack of benefit from oxime therapy is not unexpected. Parathion, diazinon and phenkaptone are diethyl phosphates, inhibition by whose active metabolites would become irreversible only relatively slowly[26,27]. Cholinesterase reactivation and beneficial effects found after oxime therapy in poisoning by these compounds were therefore to be expected. The lower effectiveness of oxime therapy with diazinon than with parathion and phenkaptone may be connected with the much slower onset and development of toxic effects with this compound, suggesting slower formation and greater persistence of the anticholinesterase metabolite, with a greater degree of irreversible inhibition[28]. With the dimethyl phosphates dimethoate and parathion-methyl the governing factor is almost certainly the rate of production and persistence of the active metabolite, controlling the amount of irreversible inhibition produced[28]. With parathion-methyl, onset and recovery were rapid, suggesting a relatively small proportion of irreversible inhibition compared with dimethoate. This is consistent with the early beneficial effect on mortality, and with the later absence of significant cholinesterase reactivation after the remaining inhibition had had time to become irreversible. The delayed recovery after oxime therapy with parathion-methyl can probably be explained on a similar basis. With dimethoate, on the other hand, onset and recovery were much slower, and so the active metabolites were apparently produced much more slowly and were more persistent, inhibition thus being almost entirely irreversible[28], and oxime therapy therefore of no benefit. This is thus a more extreme but similar effect to that with demeton-methyl, where oxime therapy was of relatively little benefit[23].

The finding that oxime therapy alone was of no apparent benefit in dimethoate poisoning then raised the question of whether it increased the value of atropine therapy, as occurs with many compounds[1,3,4,6,8,9,10,12,13,16,18,19,23]. The results summarised in Table IV suggested that it did not. It thus appears likely that synergism between oximes and atropine, as well as reactivation by oximes, depends on the enzyme inhibition being reversible, as expected. There was, in fact, a suggestion from the experiments of Table IV that administration of PAM in addition to atropine in dimethoate poisoning in the rat may possibly have been deleterious under these conditions, giving slightly higher mortality and less complete control of urinary incontinence. However, Dr. J. M. Barnes has seen no similar effect in parallel tests where no oxime therapy was given until toxic effects

had developed. In other reported work on parathion and endothion there was some indication of a slight increase in mortality of atropinised poisoned mice when the total PAM dose was increased from 50 to 100 mg./kg.[23]. Such findings may possibly be connected with the belief that low concentrations of PAM may potentiate the action of acetylcholine at nerve endings, and so possibly increase the toxic effects produced by acetylcholine accumulation[33]. In addition, there was some indication of an increase in the toxicity of PAM in the presence of atropine, but the effects were readily distinguished from those of anticholinesterase poisoning. This change is opposite from that found in the rat with P2S[31].

With the phosphorus compounds used in this investigation with rats, there was very little difference between the beneficial effects of TMB4 and of PAM at four times the TMB4 dosage. The only apparent differences were that TMB4 gave more cholinesterase reactivation with diazinon and possibly less reduction of symptom intensity with phenkaptone. TMB4 has the advantage of greater water solubility than PAM, but is more toxic. The absence of any whole brain cholinesterase reactivation with either oxime is consistent with previous results[1,9,15,16,19].

It is clear from the present study and other work that the oximes present a potentially valuable addition to the use of atropine in the treatment of poisoning by organophosphorus insecticides. It is equally clear that their therapeutic value is likely to vary considerably according to differences in the chemical structure and consequent enzyme-phosphorylating properties of organophosphates. Although with some compounds the therapeutic value of oximes may be nil, and deliberate prophylactic injection to animals may even cause adverse effects with some compounds, there appears to be no contraindication to the therapeutic use of PAM, for example, in emergency treatment of any case of human poisoning by organophosphates. There is, of course, no way of prophesying whether the results obtained in the rat are likely to apply to man, but it is known that very satisfactory results follow the use of PAM in human poisoning by parathion and parathion-methyl (Sumitomo, personal communication).

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REFERENCES

1. Davies and Green, *Brit. J. industr. Med.*, 1959, **16**, 128.
2. Hobbiger, O'Sullivan and Sadler, *Nature, Lond.*, 1958, **182**, 1498.
3. Hobbiger and Sadler, *ibid.*, 1958, **182**, 1672.
4. Poziomek, Hackley and Steinberg, *J. org. Chem.*, 1958, **23**, 714.
5. Berry, Davies and Green, *Biochem. J.*, 1959, **71**, 15P.
6. Bay, Krop and Yates, *Proc. Soc. exp. Biol., N.Y.*, 1958, **98**, 107.
7. Wilson and Ginsburg, *Biochem. Pharmacol.*, 1958, **1**, 200.
8. Berry, Davies and Green, *Brit. J. Pharmacol.*, 1959, **14**, 186.
9. Hobbiger and Sadler, *Brit. J. Pharmacol.*, 1959, **14**, 192.
10. Wills and Brown, *Armed Forces Chemical Journal*, 1957, March–April.
11. Wilson and Ginsburg, *Biochem. Biophys. Acta*, 1955, **18**, 168.

12. Wills, Kunkel, Brown and Groblewski, *Science*, 1957, **125**, 743.
13. Kewitz, Wilson and Nachmansohn, *Arch. Biochem. Biophys.*, 1956, **64**, 456.
14. Kewitz, *Ibid.*, 1957, **66**, 263.
15. Kewitz and Nachmansohn, *Ibid.*, 1957, **66**, 271.
16. Hobbiger, *Brit. J. Pharmacol.*, 1957, **12**, 438.
17. Namba and Hiraki, *J. Amer. med. Ass.*, 1958, **166**, 1834.
18. Askew, *Brit. J. Pharmacol.*, 1956, **11**, 417.
19. Askew, *ibid.*, 1957, **12**, 340.
20. Porter, Rydon and Schofield, *Nature, Lond.*, 1958, **182**, 927.
21. Rydon, *ibid.*, 1958, **182**, 928.
22. Koelle, *Science*, 1957, **125**, 1195.
23. Fournel, *C.R. Soc. Biol., Paris*, 1958, **151**, 1373.
24. Wilson and Sondheimer *Arch. Biochem., Biophys.*, 1957, **69**, 468.
25. Bethe, Erdmann, Lendle and Schmidt, *Arch. exp. Path. Pharmak.*, 1957, **231**, 3.
26. Davies and Green, *Biochem. J.*, 1956, **63**, 529.
27. Wilson, *Disc. Faraday Soc.*, 1955, **20**, 119.
28. Vandekar and Heath, *Biochem. J.*, 1957, **67**, 202.
29. Sanderson, *J. Pharm. Pharmacol.*, 1959, **11**, 150; 446.
30. Fenwick, Barron and Watson, *Biochem. J.*, 1957, **65**, 58.
31. Davies and Willey, *Brit. J. Pharmacol.*, 1958, **13**, 202.
32. Gysin and Margot, *J. agric. Food Chem.*, 1958, **6**, 900.
33. Fleisher, *Brit. J. Pharmacol.*, 1958, **13**, 288; 291.