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OXIMES AND ATROPINE IN SARIN POISONING

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Three oximes, mono*iso*nitrosoacetone (MINA), pyridine-2-aldoxime methiodide (PAM) and diacetylmonoxime (DAM), have been examined in combination with atropine as antidotes in sarin poisoning. When treatment was administered 15 min. before sarin, atropine enhanced the protective effect of MINA and DAM 2 to 3 times and of PAM 9 to 10 times in mice and rats. In mice, rats, and guinea-pigs, atropine increased by no more than 2 times the protective effect of all three oximes when given 30 sec. after sarin. Atropine given to monkeys 1 min. after sarin raised the LD50 approximately 3 times. When given in conjunction with MINA or DAM, the LD50 of sarin was raised 7 to 14 times.

It has been shown previously that two oximes, mono*iso*nitrosoacetone (MINA) and diacetylmonoxime (DAM), exert a marked protective effect in rats poisoned with *iso*propyl methylphosphonofluoridate (sarin) (Askew, 1956). Although less active in other species, they still appeared sufficiently promising to justify a further examination in conjunction with atropine. A third oxime, pyridine-2-aldoxime methiodide (PAM), the fastest known reactivator *in vitro* of cholinesterase (ChE) inhibited by sarin (Davies and Green, 1955), was found ineffective against sarin poisoning in rats (Askew, 1956), although active in mice poisoned with di*iso*propyl phosphorofluoridate (dyflos, DFP) or diethyl *p*-nitrophenyl phosphate (paraoxon) (Kewitz, Wilson, and Nachmansohn, 1956). These three oximes have now been examined as adjuvants to atropine in sarin poisoning.

METHODS

The animals used were female mice (18 to 22 g.), rats (180 to 200 g.) and guinea-pigs (320 to 380 g.), and male and female monkeys (2.5 to 4.0 kg.).

The oximes and atropine were given intraperitoneally in aqueous solution, except where otherwise stated. When atropine was given in conjunction with an oxime, the required amount of atropine was dissolved in the oxime solution immediately before use. Sarin was diluted with 0.85% saline and injected subcutaneously in a standard volume for each species. This was 10 ml./kg. for mice, 1 ml./kg. for rats and guinea-pigs, and 0.2 ml./kg. for monkeys.

LD50 values, based on a 24 hr. mortality, were determined both for sarin alone and sarin in the presence of oximes or atropine or both. For each test, the same number of animals was used at each dose, but this number varied in different experiments between four and six animals/group. The ratio between successive doses was 1.26. Since the supply of monkeys was limited, the number used varied at different dose levels, but it was never less than two ; here the ratio between doses was 1.3.

LD50 values were normally calculated by the method of moving averages (Thompson, 1947), using the tables constructed by Weil (1952). Where this was not possible the technique of probit analysis (Finney, 1947) was employed. The " relative potency " of an oxime has been expressed as the ratio of the LD50 of sarin in the presence of an oxime to the LD50 of sarin alone. The limits were obtained from the sum of the variances (V) of the individual estimates of the log. LD50, the standard error of the log. difference ($\sqrt{V}$) being multipled by the appropriate value of t at $P = 0.05$ or $P = 0.10$.

In each experiment a control determination of the LD50 of sarin was always made at the same time as that of the inhibitor plus oxime, and these values were the ones used for the calculation of relative potency. Absolute values for the LD50 in control animals varied slightly in different experiments on the same species, but, since in each experiment all dilutions were prepared from the same stock solution, estimates of relative potency are comparable from experiment to experiment.

The two oximes, MINA and PAM, were synthesized at this establishment ; DAM was obtained commercially (B.D.H.). They were all more than 95% pure.

RESULTS

The Toxicity of Sarin to Different Species.— Absolute values for the LD50 of sarin to the smaller species are given in Table I, for only

TABLE I

MEAN LD50 VALUES FOR SARIN IN DIFFERENT SPECIES

Sarin in a saline solution was injected subcutaneously.

Species	No. of Animals Used	LD50 Sarin (mg./kg.) Limits $P=0.05$
Mouse	80	0·214 (0·119–0·232)
Rat	172	0·116 (0·110–0·121)
Guinea-pig	80	0·046 (0·044–0·049)

values for the relative potency of an oxime are quoted throughout the text. These control values were obtained by combining the mortality figures from each separate estimation of sarin toxicity.

The Effect of Oximes and Atropine in Sarin Poisoning.—MINA (35 mg./kg.), PAM (100 mg./kg), DAM (41 mg./kg. and 150 mg./kg.), and atropine sulphate (50 μM./kg., namely, 17.4 mg./kg.) were given either 15 min. before or 30 sec. after the subcutaneous injection of sarin to mice, rats, and guinea-pigs. The doses of oximes used had previously been found to produce no obvious signs of poisoning in rats (Askew, 1956). The results are shown in Table II.

When MINA and atropine, or DAM and atropine, were given 15 min. before sarin, the protective effect in both rats and mice was significantly greater than when either oxime was given alone. In general, there was a 2- to 3-fold increase in effect. With PAM this increase was considerably more marked. For example, in rats the LD50 of sarin was raised only 1.3 times by either PAM or atropine when used alone, yet together they raised the LD50 approximately 14 times. A similar effect was found in the mouse.

Atropine did not enhance the action of DAM when both were given 30 sec. after sarin, although under the same conditions the protective effect of MINA was slightly increased. When PAM and atropine were given immediately following the injection of sarin, the combined effect was again greater than that of either substance used alone, but the improvement was considerably less marked than when the treatment was administered 15 min. before sarin. In rats, the effect of atropine was enhanced by PAM, although this oxime was ineffective on its own.

When given to guinea-pigs 15 min. before sarin, atropine was found to be without effect in raising the LD50 of the inhibitor, neither did it enhance the action of DAM. This may be due to its breakdown by an atropinase present in guinea-pig liver (Bernheim and Bernheim, 1938). This is consistent with the fact that atropine did exhibit some activity when given immediately following sarin.

The Combined Effect of Oximes and Atropine in Monkeys Poisoned with Sarin.—In the monkey, the oximes and atropine were given by intramuscular injection. Because of the relative insolubility of PAM (approximately 20 mg./ml.), experiments were restricted to MINA and DAM.

MINA proved more toxic to monkeys than to rats. Doses of 25 to 35 mg./kg. intramuscularly caused lethargy accompanied, in many instances, by vomiting. At 20 mg./kg., none of these side effects were obvious and this dose was therefore the maximum used in subsequent experiments. DAM was used in doses of 40 mg./kg. which again produced no obvious toxic signs and which were effective in rats. The dose of atropine (0.029 mg./kg.) was equivalent to 2 mg. to man, which is the dose recommended for use in the treatment of antiChE poisoning (Grob, 1956). Sarin was given subcutaneously into the forearm 1 min.

TABLE II

OXIMES AS ADJUVANTS TO ATROPINE IN SARIN POISONING

Sarin in saline solution was given subcutaneously and all other drugs by the intraperitoneal route. Atropine was administered in a dose of 50 μM./kg. (17·4 mg./kg.). 41 mg./kg. DAM≡35 mg./kg. MINA≡106 mg./kg. PAM.

Interval between Sarin and Other Drug(s)	Oxime	Species	Dose (mg./kg.)	Relative Potency. Limits $P=0.05$		
				Atropine Alone	Oxime Alone	Oxime+Atropine
15 min. before	MINA	Rat	35	1·3 (0·96–1·7)	4·6 (3·4–6·3)	11·6 (8·9–15·3)
	PAM	Mouse	100	1·2 (0·87–1·6)	1·4 (1·2–1·7)	11·3 (8·5–15·2)
		Rat	100	1·3 (0·96–1·7)	1·3 (0·96–1·8)	13·8 (9·9–19·2)
	DAM	Mouse	150	1·2 (0·87–1·6)	1·8 (1·4–2·3)	2·5 (2·0–3·2)
		Rat	41	1·3 (0·96–1·7)	5·3 (4·1–6·8)	9·0 (6·3–12·9)
			150		25·8 (19·3–34·4)	48·3 (34·6–67·4)
		Guinea-pig	150	1·0 (—)	2·5 (2·0–3·1)	2·6 (2·0–3·3)
30 sec. after	MINA	Rat	35	1·4 (1·1–1·7)	3·5 (2·6–4·6)	4·8 (3·8–5·9)
		Guinea-pig	35	1·3 (1·1–1·5)	1·7 (1·3–2·0)	2·7 (2·1–3·6)
	PAM	Mouse	100	—	1·3 (0·98–1·6)	1·8 (1·5–2·3)
		Rat	100	1·5 (1·1–1·9)	1·0 (0·79–1·3)	2·1 (1·7–2·5)
		Guinea-pig	100	1·3 (1·1–1·5)	1·8 (1·4–2·2)	3·1 (2·3–4·0)
	DAM	Rat	41	1·4 (1·1–1·7)	4·0 (3·1–5·2)	3·8 (3·0–4·8)
			150		8·3 (6·3–11·0)	8·0 (6·0–10·6)

before the oximes and atropine, which were injected into the thigh. The results are shown in Table III.

TABLE III

THE EFFECT OF ATROPINE ALONE AND WITH MINA OR DAM IN MONKEYS POISONED WITH SARIN

Sarin (0.2 ml. saline/kg.) was injected subcutaneously. Other injections were given intramuscularly 1 min. after sarin. Atropine was given in a dosage of 0.029 mg./0.2 ml./kg.

Treatment	LD50 Sarin (+ Treatment) (mg./kg.) Limits $P=0.10$	Relative Potency Limits $P=0.10$
None	0·038 (0·027–0·053)	1·0
Atropine	0·114 (0·082–0·156)	3·0 (2·3–4·1)
Atropine+MINA (10 mg./0·2 ml./kg.)	0·255 (0·205–0·332)	6·8 (5·2–9·0)
Atropine+MINA (20 mg./0·2 ml./kg.)	0·524 (0·422–2·16)	14·0 (9·6–20·4)
Atropine+DAM (40 mg./0·67 ml./kg.)	0·330 (0·108–0·571)	8·8 (6–12·7)

After an LD50 dose of sarin, given subcutaneously, signs of poisoning in the monkey appeared within 4 to 6 min., followed about 2 min. later by collapse and a marked decrease in respiratory rate, and death, if it occurred, was usually within 15 min. of the injection.

Although atropine raised the LD50 of sarin by approximately 3 times, recovery in survivors was slow and was not accelerated by the addition of DAM, for, although the combined treatment raised the LD50 about 9 times, the survivors were still in a state of collapse after 6 hr. Atropine combined with MINA (10 mg./kg.) increased the LD50 by almost 7 times and 5 out of 9 survivors were able to stand within 6 hr. of the initial injections. When the dose of MINA was 20 mg./kg., 9 of 11 survivors had reached the same stage of recovery within 3 hr. and the LD50 was raised by approximately 14 times.

DISCUSSION

In general, it has been shown that the protective effect of MINA, PAM, and DAM in sarin poisoning was increased when these oximes are given in conjunction with atropine. Thus, when given 15 min. before sarin, atropine gave a 2- to 3-fold increase in effect over that obtained with either MINA or DAM alone. With PAM, this increase was considerably more marked, the combined action of atropine and PAM being 9 to 10 times greater than that of the oxime itself.

There was a marked reduction in effect both of oximes alone and of oximes and atropine when they were given immediately after the inhibitor. This is probably associated with the very rapid onset of cholinergic signs following subcutaneous sarin. For example, in the rat signs of poisoning appear within 1 min. of receiving 4 times the LD50 dose of sarin, the time to death being approximately 3 min.

Although oximes have been shown to react *in vitro* with antiChE compounds and also to reactivate ChE inhibited by them (Childs, Davies, Green, and Rutland, 1955; Green and Saville, 1956), it is not yet certain how their activity *in vivo* is related to their behaviour *in vitro*.

For example, PAM reacts rapidly *in vitro* with sarin and, in addition, is the fastest reactivator of ChE when inhibited by sarin. It was expected therefore to show a fairly marked activity against sarin poisoning *in vivo*. However, it proved to be considerably less effective than either MINA or DAM in the rat, although the former is slightly less active than PAM *in vitro* whilst DAM only shows approximately one-fifth the activity of MINA.

A possible explanation of this anomaly between the *in vitro* and *in vivo* results may be provided by a consideration of the physical properties of PAM. This oxime is relatively insoluble in water and in contrast to MINA and DAM is likely to be even more lipid-insoluble since PAM, unlike MINA or DAM, cannot be extracted from aqueous solutions by either benzene or chloroform. Thus it is possible that PAM may not be readily distributed to all the main sites of ChE activity in the animal, particularly to the central nervous system.

Death from sarin poisoning in the rat appears to be due to the almost simultaneous development of central respiratory failure and neuromuscular block (Holmes, personal communication). The relative ineffectiveness of PAM when used alone in the rat could therefore be explained if this oxime were unable to penetrate centrally to any great extent. As atropine antagonizes the central effects of antiChE, the marked increase in efficacy of PAM when given together with atropine could be accounted for if the PAM acts peripherally whilst the atropine counteracts the central effects of sarin.

To test the theory that the action of PAM is restricted to peripheral sites only, experiments were carried out by J. P. Rutland, of this department, on the reactivation *in vivo* by PAM of blood and brain ChE of rats poisoned with sarin. When PAM was administered to rats 1 min. after the onset of cholinergic signs following a subcutaneous injection of sarin, reactivation of blood ChE occurred, but no such reactivation of brain ChE could be demonstrated. MINA and DAM, however, do penetrate to the central nervous

system, for quite significant increases in enzyme activity of the brain occur *in vivo*.

In the quest for a suitable antidote to antiChE poisoning, such factors as solubility, stability, and inherent toxicity of the compound must be considered. PAM is relatively non-toxic and can be given in doses up to 100 mg./kg. to the smaller species without apparent harmful side effects. The maximum dose of MINA which can be given to monkeys without producing toxic signs is 20 mg./kg. Nevertheless, with atropine, 10 mg./kg. of MINA will raise the LD50 of sarin about 7 times.

PAM is much less soluble in water than MINA and this makes its use impracticable in larger species at 100 mg./kg., the dose which has been shown to be effective in this work. Tests have not yet been carried out to determine the minimum dose of PAM which will exert a useful effect in sarin poisoning, although Kewitz *et al.* (1956) have shown that somewhat lower doses are effective against dyflos and paraoxon poisoning in mice.

DAM is less toxic than either MINA or PAM and can therefore be given at somewhat higher doses. However, it is only a poor reactivator of ChE inhibited by sarin (Childs *et al.*, 1955), a property known to be of importance in bringing signs of antiChE poisoning under control (Askew, 1956). It is therefore less likely to be of importance than MINA or PAM as a potential antidote to antiChE poisoning.

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