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2-HYDROXYIMINOMETHYL-*N*-METHYLPYRIDINIUM METHANESULPHONATE AND ATROPINE IN THE TREATMENT OF SEVERE ORGANOPHOSPHATE POISONING

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The soluble methanesulphonate of the oxime 2-hydroxyiminomethyl-*N*-methylpyridinium (P2S) has been used to treat animals poisoned with sarin or ethyl pyrophosphate. The effect of the size of the dose, and its time of administration in relation to poisoning, have been examined. This oxime is very efficient in conjunction with atropine when given either before or after poisoning. About 30 mg./kg. seems to be the optimum therapeutic dose of the methanesulphonate. The significance of this optimum is discussed in relation to the treatment of accidental poisoning by organophosphate insecticides in man.

In conjunction with atropine, the iodide of the oxime 2-hydroxyiminomethyl-*N*-methylpyridinium (pyridine-2-aldoxime methiodide; P2AM) is an effective antidote to organophosphate poisoning. Thus Kewitz, Wilson, and Nachmansohn (1956) showed that 50 mg./kg. of the iodide with 10 mg./kg. of atropine, given intraperitoneally, were effective against diethyl *p*-nitrophenyl phosphate (E600) or dyflos (di-*iso*propyl phosphorofluoridate). Askew (1957), using 100 mg./kg. of the iodide with 17.4 mg./kg. of atropine sulphate, treated successfully *iso*propyl methylphosphonofluoridate (sarin) poisoning, and Wilson and Sondheimer (1957) found a similar combination effective against poisoning by ethyl pyrophosphate. Further evidence of the potentially wide application of the iodide with atropine has been provided by Hobbiger (1957), by Bethe, Erdmann, Lendle, and Schmidt (1957), and by Fournel (1958).

The dose of the iodide used by all these authors was relatively large. Since the iodide is only about 4% soluble in water, the injection of high doses into man would require large volumes of solution. If given intravenously to rabbits in conjunction with atropine, it is highly effective against sarin poisoning in doses as low as 5 mg./kg. (Wills, Kunkel, Brown, and Groblewski, 1957). However, since convulsions are frequently present in severe anticholinesterase poisoning, intravenous injections even of this size will often be impossible. A more soluble salt of this oxime with a potency at least equal to that of the iodide, yet capable of being administered intramuscularly in small volumes of solution of sufficient strength, is thus highly desirable. Such a salt is the methanesulphonate (P2S), 1 g. of which is soluble in 2 to 3 ml. of water (Davies and Willey, 1958). In this paper, the therapeutic and prophylactic potentialities of this methanesulphonate have been assessed in the treatment of poisoning by sarin or ethyl pyrophosphate in laboratory animals.

METHODS

The methanesulphonate was prepared by boiling 2-hydroxyiminomethylpyridine with methyl methanesulphonate in benzene; it had an m.p. of 155°. For injection it was dissolved in water, *p*H being adjusted to 7.4 with 0.1 N-NaOH immediately before use. Atropine sulphate (B.D.H.) was always injected in the same solution as the oxime. The therapeutic agents were given intramuscularly in volumes of 3 ml./kg. body weight to mice, and 1 ml./kg. to rats, guinea-pigs and rabbits. Sarin or ethyl pyrophosphate was given subcutaneously in saline in volumes of 10 ml./kg. to mice, 1 ml./kg. to rats and guinea-pigs, and 0.5 ml./kg. to rabbits. Female mice (18 to 22 g.), rats (180 to 200 g.), guinea-pigs (350 to 400 g.) and rabbits (2 to 3 kg.) were used.

In general, the potency of any treatment was measured in terms of LD50 values, although in some

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prophylactic experiments it was more convenient to give a standard number of animals a fixed dose of the organophosphate at various intervals after the therapeutic agents.

Each LD50 was calculated by the method of moving averages (Thompson, 1947), using the tables compiled by Weil (1952). A four-point assay was usually performed, with six animals at each dose of organophosphate.

RESULTS

The Probit Regression Line in the Presence and Absence of Treatment.—Assessments of the potency of treatment were made by comparing the LD50 of the organophosphate in the presence and absence of treatment. This is a purely arbitrary basis of comparison. It cannot be extended to the whole dose/response curves since these were not always parallel, their slopes almost always decreasing as the treatment became increasingly effective. A few examples to illustrate this decrease are given in Table I.

TABLE I

THE PROBIT/LOG DOSE REGRESSION LINES IN SARIN AND ETHYL PYROPHOSPHATE POISONING IN THE PRESENCE AND ABSENCE OF TREATMENT WITH 2-HYDROXYIMINOMETHYL-*N*-METHYLPYRIDINIUM METHANESULPHONATE AND ATROPINE

Treatment with oxime and atropine was given 1 min. after sarin in mice, 10 min. before sarin in rabbits, 10 min. before ethyl pyrophosphate. The values for the LD50 are given with the 95% limits.

Species	Toxic Agent	Dose of Oxime (mg./kg.)	No. Animals	LD50 (mg./kg.)	Regression Coefficient ± s.d.
Mouse	Sarin	0	40	0·305 (0·27–0·37)	10·2±2·7
		30	40	1·0 (0·79–1·30)	4·8±1·1
Rabbit	,,	0	40	0·05 (0·044–0·055)	10·1±1·9
		30	50	6·53 (4·80–9·50)	3·3±0·9
Rat ..	Ethyl pyrophosphate	0	40	0·38 (0·34–0·43)	11±2·6
		120	40	5·18 (3·60–10·9)	4·2±1·6

Insufficient animals were used in most of the LD50 determinations to show that this decrease was always statistically significant; but the trend was invariably found.

A Comparison of the Methanesulphonate and Iodide of the Oxime as Adjuvants to Atropine.—As the functional part of both salts is the 2-hydroxyiminomethyl-*N*-methylpyridinium ion, the therapeutic effectiveness of these two salts would therefore not be expected to differ significantly. That this is so against sarin poisoning is confirmed by the results given in Table II.

TABLE II

COMPARISON OF THE METHANESULPHONATE AND IODIDE OF 2-HYDROXYIMINOMETHYL-*N*-METHYLPYRIDINIUM AS ADJUVANTS TO ATROPINE IN SARIN POISONING

Oxime (30 mg./kg.) with atropine sulphate (17·4 mg./kg.) was given 10 min. before sarin. The values for the LD50 for sarin are given with 95% limits.

Species	Sarin LD50 (mg./kg.)		
	No Treatment	Methanesulphonate + Atropine	Iodide + Atropine
Mouse ..	0·30 (0·23–0·42)	0·98 (0·79–1·20)	1·34 (1·05–1·72)
Rat ..	0·14 (0·09–0·18)	0·45 (0·28–0·71)	0·38 (0·27–0·54)
Guinea-pig	0·048 (0·041–0·057)	3·81 (2.66–5·48)	4·65 (3·16–6·87)
Rabbit ..	0·050 (0·046–0·055)	6·12 (4·41–8·49)	6·54 (5·00–8·60)

The Therapeutic Efficacy of the Methanesulphonate with Atropine.—In these experiments "therapy" refers to treatment after poisoning and "prophylaxis" to treatment before poisoning. Table III shows the effect of 30 mg./kg. of the methanesulphonate combined with 17.4 mg./kg. of atropine sulphate, given 1 min. after sarin or ethyl pyrophosphate, in mice, rats, guinea-pigs and rabbits.

TABLE III

THE "THERAPEUTIC" ACTIVITY OF 2-HYDROXYIMINOMETHYL-*N*-METHYLPYRIDINIUM METHANESULPHONATE (30 MG./KG.) WITH ATROPINE SULPHATE (17·4 MG./KG.) AGAINST SARIN AND ETHYL PYROPHOSPHATE

Treatment was given intramuscularly 1 min. after the subcutaneous injection of toxic agent. The LD50 values are given with the 95% limits in mg./kg.

Species	LD50 of Toxic Agent Alone		LD50 of Toxic Agent in Treated Animals	
	Sarin	Ethyl Pyrophosphate	Sarin	Ethyl Pyrophosphate
Mouse ..	0·30 (0·22–0·42)	0·58 (0·52–0 64)	1·2 (0·73–2·2)	11·7 (9·1–15·2)
Rat ..	0·14 (0·09–0·18)	0·38 (0·34–0·43)	0·22 (0·16–0·32)	2·2 (1·6–3·2)
Guinea-pig	0·048 (0·041–0·057)	0 70 (0·37–1·3)	1·8 (1·3–2·5)	14·0 (8·7–23)
Rabbit ..	0 050 (0·046–0·055)	0·30 (0·27–0·32)	2·0 (1·6–2·5)	6·3 (5·1–7·9)

This treatment was very effective against ethyl pyrophosphate in each species and against sarin in rabbits and guinea-pigs. Rats and mice poisoned with sarin were less responsive to treatment. The effect of varying the dose of methanesulphonate, while keeping that of atropine constant, is shown in Table IV. In mice the increase in therapeutic effect with increasing dose of methanesulphonate fell off rapidly once the dose exceeded 30 mg./kg. This tendency was less marked in rats.

TABLE IV

THE EFFECT OF DOSE UPON THE "THERAPEUTIC" POTENCY OF 2-HYDROXYIMINOMETHYL-*N*-METHYLPYRIDINIUM METHANESULPHONATE IN THE PRESENCE OF A CONSTANT DOSE OF ATROPINE

The dose of atropine sulphate was 17·4 mg./kg. The LD50 values are given with 95% limits in mg./kg. 0 indicates that neither oxime nor atropine was given.

Toxic Agent	Methanesulphonate (mg./kg.)	LD50	
		Rats	Mice
Sarin ..	0	0·14 (0 09–0·18)	0·30 (0·22–0·42)
	15	—	0·93 (0·58–1·5)
	30	0·22 (0·16–0·32)	1·20 (0·73–2·2)
	120	0·59 (0·34–1·0)	1·13 (0·79–1·6)
Ethyl pyrophosphate	0	0·38 (0·34–0·43)	0·58 (0·52–0·64)
	10	1·8 (1·1–2·9)	2·8 (1·7–4·6)
	30	2·2 (1·6–3·2)	11·7 (9·1–15·2)
	120	5·0 (3·6–7·1)	11·3 (7·1–18·0)

The Prophylactic Efficacy of the Methanesulphonate with Atropine

The protective effect of the methanesulphonate (30 mg./kg.) with atropine sulphate (17.4 mg./kg.) given 10 min. before poisoning is shown in Table V. In general, treatment before poisoning was more effective than treatment after poisoning. The LD50 of ethyl pyrophosphate was raised 20- to 50-fold in each species; the LD50 of sarin was increased 70 to 100 times in rabbits and guinea-pigs, but only 3 to 4 times in rats and mice. In contrast to the "therapeutic" experiments, the prophylactic effect of the methanesulphonate increased approximately linearly with increasing dose in both rats and mice (see Table VI).

The Influence of the Interval Between Treatment and Poisoning

The two purposes of these experiments were, first, to determine how long the protective action of a given dose of the methanesulphonate lasted, and, second, to see whether there was an optimum time between treatment and poisoning. The maximum dose of the methanesulphonate that could be given without observable side-effects was 120 mg./kg. (Davies and Willey, 1958). At this dose some protection was still obtained in rats, even when the organophosphate was given more than 3 hr. after treatment; in mice, however, protection had almost disappeared after less than 2 hr. (Table VII). The duration of the protective effect fell off fairly rapidly as the dose of the methanesulphonate was decreased. The optimum interval between treatment and poisoning was greater at the highest dose of the methanesulphonate, but a 10 min. interval almost always gave nearly optimal results. Very similar results were obtained in a few experiments with ethyl pyrophosphate.

TABLE V

THE "PROPHYLACTIC" ACTIVITY OF 2-HYDROXYIMINOMETHYL-*N*-METHYLPYRIDINIUM METHANESULPHONATE (30 MG./KG.) WITH ATROPINE SULPHATE (17·4 MG./KG.) AGAINST SARIN AND ETHYL PYROPHOSPHATE

Treatment was given intramuscularly 10 min. before the subcutaneous injection of the toxic agent. The LD50 values with 95% limits are given in mg./kg.

Species	LD50 of Toxic Agent Alone		LD50 of Toxic Agent in Treated Animal	
	Sarin	Ethyl Pyrophosphate	Sarin	Ethyl Pyrophosphate
Mouse ..	0·30 (0·22–0·42)	0·58 (0·52–0·64)	1·1 (0·79–1·9)	20 (16–25)
Rat ..	0·14 (0·09–0·18)	0·38 (0·34–0·43)	0·45 (0·28–0·71)	17 (7·8–30)
Guinea-pig	0·048 (0·041–0·050)	0·70 (0·4–1·3)	3·5 (2·3–5·5)	35 (20–60)
Rabbit ..	0·050 (0·046–0·055)	0·30 (0·27–0·32)	5·1 (3·0–8·5)	8·4 (6·0–11·6)

TABLE VI

THE EFFECT OF DOSE UPON THE "PROPHYLACTIC" POTENCY OF 2-HYDROXYIMINOMETHYL-*N*-METHYLPYRIDINIUM METHANESULPHONATE IN THE PRESENCE OF A CONSTANT DOSE OF ATROPINE

The dose of atropine sulphate was 17·4 mg./kg. The LD50 values with 95% limits are given in mg./kg. 0 inicates that neither oxime nor atropine was given.

Toxic Agent	Oxime mg./kg.	LD50	
		Rats	Mice
Sarin	0	0·14 (0·09–0·18)	0·30 (0·22–0·42)
	30	0·45 (0·28–0·71)	1·1 (0·79–1·9)
	120	1·4 (1·1–1·9)	5·0 (3 8–6·6)
Ethyl pyrophosphate	0	0·38 (0·34–0.43)	0·58 (0·52–0·64)
	10	2·5 (2·0–3·2)	5·3 (4·0–7·0)
	30	17 (7·8–30)	20 (16–25)
	90	—	37 (26–64)
	120	36 (22–57)	95 (66–138)

TABLE VII

EFFECT OF TIME BETWEEN ADMINISTRATION OF VARIED DOSES OF 2-HYDROXYIMINOMETHYL-*N*-METHYLPYRIDINIUM METHANESULPHONATE AND ATROPINE AND INJECTION OF SARIN

Atropine sulphate, 17·4 mg./kg., was given. Dose of sarin in rats, 0·75 mg./kg. (5·4 × LD50); and in mice, 1·2 mg./kg. (4.0 × LD50). Numerals in table give mortalities in groups of 25 animals.

Methanesulphonate mg./kg.	Time (min.)						
	200	100	50	10	5	−1	−5
Rats							
120	19	6	3	11	11	15	25
60	—	21	15	16	12	23	—
30	—	23	15	18	15	23	—
15	—	—	23	20	19	24	—
Mice							
120	—	23	1	4	6	12	25
60	—	25	22	5	10	8	—
30	—	—	23	17	13	17	—
15	—	—	23	14	14	19	—

DISCUSSION

In this paper the efficacy of the methanesulphonate of 2-hydroxyiminomethyl-*N*-methylpyridinium with atropine in the therapeutic and

prophylactic treatment of animals poisoned with sarin and ethyl pyrophosphate has been reported. When the methanesulphonate (30 mg./kg.) and atropine were given therapeutically, the LD50 of ethyl pyrophosphate was raised 20-fold in mice, guinea-pigs and rabbits, although only 5-fold in rats. The LD50 of sarin was raised 40-fold in guinea-pigs and rabbits, but only about 4-fold in mice, and still less in rats. Except in rats poisoned with sarin, increasing the dose above 30 mg./kg. produced no proportionate increase in effect. Prophylactically this limiting effect of increased dose was not apparent: the consequence of increasing the dose was particularly striking on the duration of the prophylactic effect (see Table VII). A similar species variation was noticed as in the therapeutic experiments, especially in the resistance to treatment of rats and mice poisoned with sarin. This anomaly in the behaviour of rats was particularly striking in the light of earlier therapeutic experiments with the oximes hydroxyiminoacetone and diacetyl monoxime (Askew, 1956, 1957). These two compounds, especially the latter, were far more effective against sarin poisoning in rats than in any other species.

The apparent optimal therapeutic dose of 30 mg./kg. may be related to the fact that reactivation by the methanesulphonate reached a maximum rate at quite low concentrations (Green and Smith, 1958). Prophylactically, the higher doses might enable a fairly persistent optimal concentration to be maintained in the body tissues.

The value of these results depends upon the extent to which they can be applied to man, and here factors other than the intrinsic potency of the oxime and atropine are important. For example, the very high water solubility of the methanesulphonate gives it a distinct advantage over the iodide in ease of administration. In accidental insecticide poisoning, therapy will be more important than prophylaxis, and in these circumstances the possible existence of a relatively low optimal single dose (30 mg./kg.) of the methanesulphonate is decidedly helpful. The administration of a dose of this magnitude intramuscularly to human beings, about 2 g./man, would probably be a practical field procedure.

Namba and Hiraki (1958) have, in fact, successfully given 1 g. of the iodide (in 20 ml. water) intravenously to agricultural workers accidentally poisoned with parathion; but in very severe poisoning accompanied by convulsions, though intravenous injections would be impossible, intramuscular injections would still be practicable.

As a prophylactic measure, the regular injection of the methanesulphonate is unlikely to be widely applicable. In rats and mice even large doses, of more than 100 mg./kg., only provide substantial protection for about 2 hr. However, recent experiments seem to show that a satisfactory form of "prophylactic" treatment in rats is to give the oxime orally and prophylactically, and the atropine intramuscularly and therapeutically (Creasey, personal communication).

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