SELF-ADMINISTRATION OF PRALIDOXIME IN NERVE GAS POISONING WITH A NOTE ON THE STABILITY OF THE DRUG

By Rolf Barkman, Bo Edgren and Anders Sundwall

From the Central Military Pharmacy and the Research Institute of National Defence, Department 1, Stockholm, Sweden

Received April 19, 1963

Plasma concentrations of pralidoxime methane sulphonate (P2S) have been determined in man after self-administration by an automatic injector. After administration of 10 mg./kg. P2S in a 50 per cent w/v solution, concentrations over 4 μg./ml. were reached within 6 min. and maintained for about 90 min. After 20 mg./kg., plasma concentrations over 4 μg./ml. were maintained for about 170 min. Except for pain at the site of injection, which disappeared after a few hr., no serious side reactions could be detected. The drug can be stored for 5 years at 5° with less than 7 per cent decomposition. The decomposition products were found to be less toxic than the unchanged oxime. *N*-Methylpyridinium-2-carboxamide was identified as a major degradation product.

Certain oximes, when used in combination with atropine, provide an effective therapy in animals exposed to large doses of organophosphorous cholinesterase inhibitors, such as diethyl 4-nitrophenyl thionophosphate (parathion), tetraethylpyrophosphate (TEPP) and methyl-isopropoxy-phosphoryl fluoride (sarin) (see Sundwall, 1962). The oximes act by reactivating the phosphorylated cholinesterase and thus provide a causal therapy (Childs, Davies, Green and Rutland, 1955; Wilson and Ginsburg, 1955). One of the most potent oximes, *N*-methylpyridinium-2-aldoxime (pralidoxime) has been used in man in both experimental and accidental organophosphate poisoning (Grob and Johns, 1958; Namba and Hiraki, 1958; Karlog, Nimb and Poulsen, 1958). However, after exposure to large doses of nerve gases there is a little delay before severe symptoms appear. The efficacy of treatment will therefore depend very much on the interval of time between exposure and treatment with antidotes. Rapid treatment is also necessitated by the ageing of the phosphorylated enzyme. These considerations mean that a method of self-treatment and first aid is highly desirable, particularly in the event of chemical warfare.

The possibility of administering the drugs by automatic injectors has been discussed (Barkman, 1960), but insufficient information has been available concerning the practicability of this proposition. It is not known if it is possible, with an automatic injector, to administer the relatively large doses that generally have been considered necessary for a reliable therapeutic effect. Nor is it certain whether pralidoxime can be stored for a long time in aqueous solutions, because of instability (Creasy and Green, 1959; Halse and Skogan, 1959.

The aim of the present investigations was to determine the plasma concentrations reached with the Astra auto-injector previously described (Barkman, 1959) and to test the stability of the drug.

METHODS

Intramuscular Administration of Pralidoxime

The injections were made with an automatic injector as described by Barkman (1960) (see Fig. 1). The injector was loaded with 1·5 ml. of a

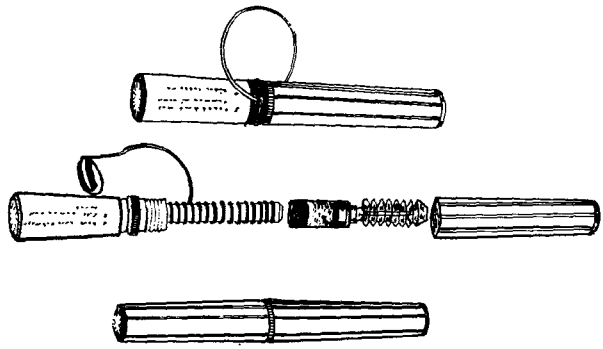

FIG. 1. Above: Closed view of the injector. Middle: Exploded view of the injector. The ring and loop function as a safety catch. Below: Case with two spare needles and ampoules attached.

50 per cent w/v aqueous solution of pralidoxime methane sulphonate (P2S). The solutions were filled in the plastic ampoules of the injector and sterilized by autoclaving at 120° for 20 min. This procedure produces about 5 per cent loss of oxime (Barkman, 1959), without influencing the acute toxicity of the solution. The experiments were done on 24 healthy human volunteers of both sexes (age 21 to 35 years). The injections were administered by the test persons themselves on the outside of the thigh. Blood samples were taken at intervals from an antecubital vein and the plasma analysed for P2S by an ultra-violet method (Sundwall, 1960).

Long-term Storage of P2S in Aqueous Solutions

The pH for maximum stability at 120° was about 3 when measured at 25° (Barkman, 1959). For this reason solutions of P2S (16 per cent w/v) were buffered at pH 2·5, 3·0 and 3·5 although the pH of maximum stability is 4 at room temperature. The solutions were stored in sealed glass ampoules at 5, 15, 25 and 45° for 2 years. The buffer solutions were prepared from 0·2M disodium phosphate and 0·1M citric acid, according to McIlvaine and Sörensen.

The solutions were analysed for unchanged pralidoxime after 1, 2, 3, 6, 12 and 24 months by an ultra-violet procedure (Ellin and Kondritzer, 1959). The precision of this method under the experimental conditions used is ± 2 per cent (Barkman, 1959). The specificity of the method has been examined by Ellin (1958) and by Ellin and Kondritzer (1959), who found that neither acid nor alkaline hydrolysis of pralidoxime produces substances that interfered. Hydroxylamine and hydrogen cyanide are initially formed during breakdown of P2S (Creasy and Green, 1959; Ellin and Kondritzer, 1959). Hydroxylamine was determined colorimetrically with a diazo reaction after conversion to nitrite (Csáky, 1948)

and hydrogen cyanide with a modified Zincke-König reaction (Asmus and Garschagen, 1953). To be able to determine eventual hydrogen cyanide, the ampoules used were broken in a plastic test-tube together with N sulphuric acid. The liberated hydrocyanic acid was absorbed in 0·1N sodium hydroxide and determined.

The decomposition products were separated by descending paper chromatography with n-butanol:acetic acid:water (5:1:3) (Ellin and Easterday, 1961). The spots were examined under an ultra-violet lamp and by spraying with a modified Dragendorff spray reagent (Ellin and Easterday, 1961). *N*-Methylpyridinium-2-nitrile methane sulphonate and *N*-methylpyridinium-2-carboxyamide iodide were synthesized by the method described in the literature (Enander, Sundwall and Sörbo, 1961, 1962).

Acute toxicity of the solutions using the intraperitoneal route of injection (10 ml./kg.) was determined in male and female mice (20 ± 1 g.). LD50 calculations were made by the method of Miller and Tainter (1944).

Results and Discussion

Intramuscular Administration of P2S with the Automatic Injector

In a previous investigation, 8 to 10 ml. of 25 per cent (30 mg./kg. body weight) aqueous solutions of P2S were injected intramuscularly in human volunteers without severe pain or other signs of severe local reactions (Sundwall, 1960).

In the present investigation, 1·5–3·0 ml. of a 50 per cent aqueous solution of the drug was used.

TABLE I

Rate of absorption and maintenance of therapeutic plasma concentrations of P2S after different doses and routes of administration in man

Number of experiments	Dose of oxime	Time after which conc. of P2S reached 4 μg./ml. ± s.e. (min.)	Length of time which conc. of above 4 μg./ml. was maintained ± s.e. (min.)
12	0·75 g. i.m.	5·9 ± 1·1	90 ± 12
4	2 × 0·75 g. i.m.	4·3 ± 1·3	169 ± 13
6	0·75 g. i.m. + 2 g. orally	3·8 ± 0·7	208 ± 28
3	0·75 g. i.m. followed by 2 g. orally after 30 min.	6·7 ± 0·9	200 ± 17

Twelve experiments were made using one injection corresponding to about 10 mg./kg. body weight, and four experiments with two injections, about 20 mg./kg., given within 1 min. After most injections, the subjects complained of pain at the site of the injection. This was most pronounced in those who were physically trained. The pains disappeared within a few hr. The results of the absorption studies are summarised in Fig. 2 and Table I. As can be seen, this form of administration gives a rapid absorption. The maximum plasma concentrations were reached

within 20 min. The peak plasma concentrations after two injections are roughly double those after a single injection. Earlier experiments indicated that plasma concentrations over 4 μg./ml. are needed for any therapeutic effect (Sundwall, 1961). This concentration was reached

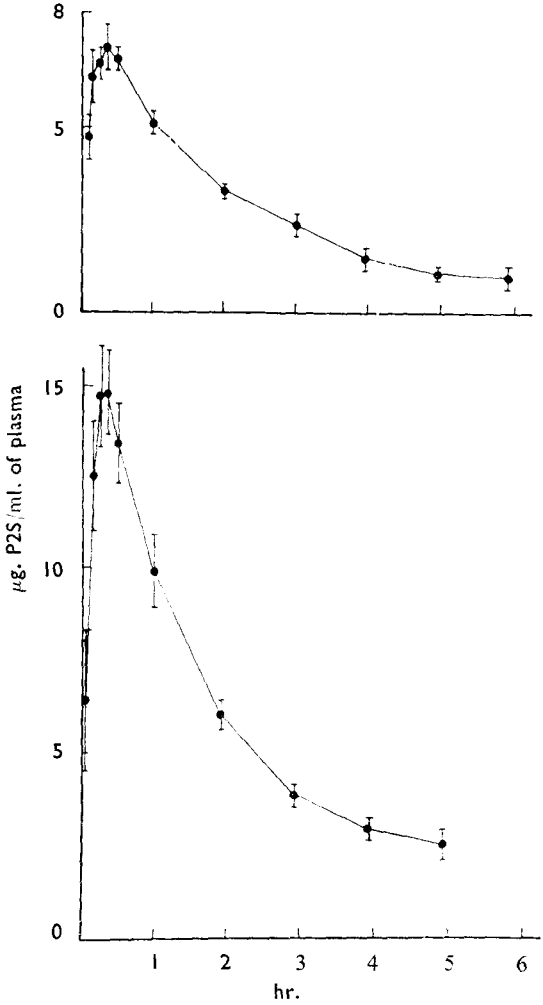

FIG. 2. Plasma concentrations of P2S in man following self administration with an automatic injector. Above: One injection (about 10 mg./kg.). Below: Two injections within 1 min. (about 20 mg./kg.).

within 4–6 min. (see Table I) and was maintained for about 1½ hr. after a single injection and nearly twice that time after two injections.

A combination of intramuscular and oral administration was then tested, to see if rapid absorption and prolonged maintenance of therapeutic plasma concentrations could be obtained (Fig. 3). Table I shows

that this combination gave no better results than two intramuscular injections.

No serious side reactions were noticed. About one third of the subjects complained of drowsiness but this effect is difficult to evaluate as no blind test was made. One person experienced diplopia for a few min. after two injections.

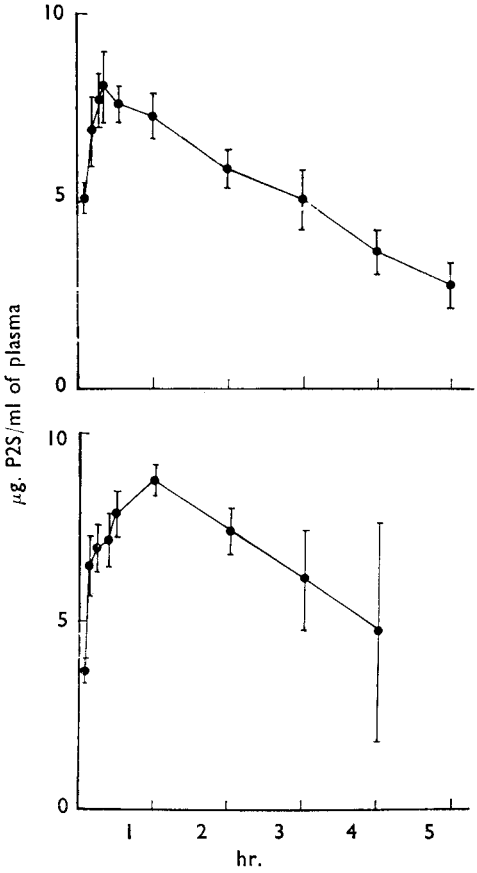

FIG. 3. Plasma concentrations of P2S in man after a combination of self-injection and administration by mouth. Above: One injection and 2 g. P2S orally within 1 min. (10 mg./kg. and 30 mg./kg. respectively). Below: One injection followed by oral administration of 2 g. P2S 30 min. later (10 and 30 mg./kg. respectively.

In animal experiments, myositis has been described after repeated intramuscular injections (Albanus, Järplid and Sundwall, 1961). Special attention was therefore paid to the possibility of necroses at the site of injection but no signs could be detected.

Long-term Storage of P2S in Aqueous Solution

The unchanged amounts of P2S remaining in buffered solutions of pH 2·5, 3·0 and 3·5 after 2 years storage at different temperatures are

summarised in Table II. At temperatures of 5, 15 and 25° there is less than 6 per cent deterioration of pralidoxime. But there was much decomposition at 45°.

All solutions were analysed for hydrogen cyanide and hydroxylamine which were present in amounts corresponding to about 0·05 and 0·3 per cent respectively.

Paper chromatography of the solutions with 80 per cent decomposition revealed that the two main decomposition products had the same R_F values in acetic acid : n-butanol : water (5 : 1 : 3) as authentic samples of *N*-methylpyridinium-2-carboxyamide and *N*-methylpyridinium-2-nitrile (R_F 0·36 and 0·41 respectively). The amide, not previously identified as a degradation product of pralidoxime, was identified as follows. The presumed amide spot was eluted and re-chromatographed in another solvent system, 95 per cent ethanol : strong ammonia (s.g. 0·880) (19 : 1), together with marker amide. The spots which were localised with the modified Dragendorff spray reagent were found to have the same R_F value (0·29). This solvent system resolves *N*-methylpyridinium-2-carboxylic acid from *N*-methylpyridinium-2-carboxyamide (Enander and others,

TABLE II

UNCHANGED PRALIDOXIME, PER CENT, AFTER TWO YEARS STORAGE IN BUFFERED WATER SOLUTIONS (16 PER CENT W/V) AT pH 2·5–3·5 FROM 5–45°

Temperature °C.	Per cent unchanged		
	pH 2·5	pH 3·0	pH 3·5
5	98·4	98·4	97·8
15	98·1	97·0	96·3
25	95·7	96·0	94·0
45	55·0	25·0	20·0

1962). In another experiment, the spots of the unknown and authentic samples were eluted and their ultra-violet absorption spectra compared in 0·1N sodium hydroxide. The same spectrum was found.

No difference in the acute toxicity could be demonstrated between freshly prepared solutions and the solutions stored at between 5 and 25° (LD50, 125 mg./kg.). The solutions stored at 45° with an 80 per cent decomposition were considerably less toxic (LD50 about 400–500 mg./kg.) The LD50 of the amide was over 400 mg./kg.

From practical and economic considerations it is essential to store P2S for at least 5 year periods. It is of fairly minor importance, however, if 10 per cent active material is lost after storage, provided that toxic decomposition products are not produced. Our experiments suggest that after 5 years storage at 5° the decomposition should be less than 7 per cent. The acute toxicity of the more decomposed solutions reveals that the decomposition products are less toxic than the parent compounds. Results in agreement with our stability tests have recently been published by Kondritzer and others (1961) for pralidoxime chloride.

REFERENCES

Albanus, L., Järplid, B. and Sundwall, A. (1961). *Acta pharm. tox. Kbh.*, **18**, 321–328.
Asmus, E. and Garschagen, H. (1953). *Ztschr. Anal. Chem.*, **138**, 414–422.
Barkman, R. (1960). *Tidskrift i militär hälsovård*, **85**, 1–32.
Childs, A. F., Davies, D. R., Green, A. L. and Rutland, J. P. (1955). *Brit. J. Pharmacol.*, **10**, 462–465.
Creasy, N. H. and Green, A. L. (1959). *J. Pharmacol.*, **11**, 485–489.
Csáky, Z. (1948). *Acta chem. scand.*, **2**, 450–454.
Davies, D. R., Green, A. L. and Willey, G. L. (1959). *Brit. J. Pharmacol.*, **14**, 5–8.
Ellin, R. I. (1958). *J. Amer. chem. Soc.*, **80**, 6588–6990.
Ellin, R. I. and Kondritzer, A. A. (1959). *Analyt. Chem.*, **31**, 200–201.
Ellin, R. I. and Easterday, D. E. (1961). *J. Pharm. Pharmacol.*, **13**, 370–373.
Enander, I., Sundwall, A. and Sörbo, B. (1961). *Biochem. Pharmacol.*, **7**, 232–236.
Enander, I., Sundwall, A. and Sörbo, B. (1962). *Ibid.*, **11**, 377–382.
Grob, D. and Johns, R. J. (1958). *Amer. J. Med.*, **24**, 497–515.
Halse, M. and Skogan, O. (1959). *Medd. Norsk Farm. Selsk.*, **11**, 141–157.
Karlog, O., Nimb., M. and Poulsen, E. (1958). *Ugeskr. Laeg.*, **120**, 177–183.
Kondritzer, A. A., Ellin, R. I. and Edberg, L. J. (1961). *J. pharm. Sci.*, **50**, 109–112.
Miller, L. C. and Tainter, M. L. (1944). *Proc. Soc. exp. Biol., N.Y.*, **57**, 261–265.
Namba, T. and Hiraki, K. (1958). *J. med. Ass.*, **166**, 1834–1839.
Sundwall, A. (1960). *Biochem. Pharmacol.*, **5**, 225–230.
Sundwall, A. (1961). *Ibid.*, **8**, 413–417.
Sundwall, A. (1962). *Tidskrift i militär hälsovård*, **87**, 3–31.
Wilson, I. B. and Ginsburg, S. (1955). *Biochem. Biophys. Acta*, **18**, 168–170.