Studies on the Efficacy of Diethyxime as an Antidote against Organophosphorus Intoxication in Rats

S.N. DUBE, A.K. GHOSH, K. JEEVARATHINAM, D. KUMAR, S. DAS GUPTA, B.P. PANT*, B.S. BATRA* and D.K. JAISWAL.*

Division of Pharmacology and *Division of Synthetic Chemistry, Defence Research and Development Establishment, Tansen Road, Gwalior-474002, India

Accepted March 3, 1986

Abstract—Diethyxime, a non-quaternary cholinesterase reactivator was evaluated for its antidotal efficacy against organophosphorus intoxication in rats using the protection index, cholinesterase reactivation and neuromuscular function as the experimental protocol. Diethyxime along with atropine produced a marked antidotal effect against dimethyl dichlorovinyl phosphate (DDVP) poisoning on all the parameters studied. The action of diethyxime was mainly peripheral. The protective efficacy against diisopropyl fluorophosphate (DFP) poisoning was not observed with this reactivator.

The therapy of organophosphorus (OP) intoxication commonly involves co-administration of cholinolytics, viz. atropine and cholinesterase reactivators. Amongst the latter drugs, pyridinealdoximes such as pralidoxime (2-PAM), obidoxime (LüH6) and trimedoxime (TMB-4) are known to be therapeutically most effective (1). The pyridinealdoximes have the disadvantage of limited tissue distribution. Being quaternary salts, these compounds do not readily penetrate into central nervous system, causing poor reactivation at the central site (2). Several attempts have been made therefore to design reactivators with a non-quaternary structure (3, 4). Kokshareva et al. (5) claimed diethyxime (S-[2-(diethylamino)ethyl]-4-bromobenzo-thiohydroximate hydrochloride), a non-quaternary compound, as a universal antidote having potent reactivation at central and peripheral sites against OP intoxication. However, the in vitro reactivation measurements by Kenley et al. (3) and our initial work on the protection index with diethyxime in diisopropylfluorophosphate (DFP) poisoned rats (6) did not support this observation.

In this paper, a systematic investigation on the therapeutic efficacy of diethyxime in OP intoxication with dimethyl dichlorovinyl phosphate (DDVP) and DFP is reported based on (i) in vivo cholinesterase (ChE) reactivation (ii) protection index (PI) and (iii) recovery of neuromuscular (NM) functions.

Materials and Methods

Albino rats weighing 90–100 g and 150–200 g were used for (i) PI and ChE measurements and (ii) experiments on NM function studies, respectively. The compounds DDVP, DFP and diethyxime were synthesised in the Chemistry Division of our Establishment. Their physical characteristics, IR and 1H NMR data were in agreement with the reported literature values (7–9). Purity of OP compounds was >98% checked by GLC, and diethyxime was homogenous on TLC.

Twenty-four hour LD$_{50}$ values of the OP compounds and diethyxime were determined by Dixon's Up and Down method (10). The therapeutic efficacy of diethyxime was evaluated (6) by determining PI.

Protection index (PI) = $\frac{LD_{50} \text{ of organophosphate treated with antidotes}}{LD_{50} \text{ of organophosphate}}$
The ChE activity was measured in serum, corpus striatum and diaphragm muscle. The blood was collected from retro-orbital plexuses after 1 hr of treatment, and immediately thereafter, the animals were sacrificed to dissect out the corpus striatum on ice (11) and diaphragm muscle. The dissected diaphragm was rinsed in ice-cold physiological saline before homogenization (12). A 5% (w/v) homogenate of corpus striatum and diaphragm prepared in ice-cold 0.1 M sodium phosphate buffer at pH 8.0 was centrifuged at 1600 g in a cold centrifuge for 10 min (13). The supernatant was used as the enzyme source for ChE activity measurement by Ellman’s method (14) at 28±1°C in a Pye Unicam SP-8-500 UV Visible Spectrophotometer. Protein was estimated by the Biuret method (15).

Neuromuscular studies were conducted in pentobarbitone anaesthetized rats. The trachea was opened to provide artificial respiration. The ECG was recorded using lead II electrodes. The sciatic nerve was stimulated indirectly with the help of a bipolar silver electrode with trains of stimuli at 25, 50, 100 and 200 Hz each lasting 3 sec (16) using a Grass Stimulator S88. The tetanic responses were recorded on a Physioscribe. The overall percentage NM function was determined from the four tetanic responses (17).

**Results**

Enzyme ChE activity measurements (Table 1) indicated DFP to be a more potent inhibitor of ChE enzyme than DDVP when an equitoxic dose of either OP compound was administered in rats. Diethyoxime had reactivated DDVP, and DFP inhibited ChE peripherally; the reactivation produced was statistically significant by Student’s t-test in both DDVP and DFP poisoning. However, the amount of reactivation produced was

| Groups       | Corpus striatum (Percent of control) ± S.E. | Diaphragm (Percent of control) ± S.E. | Serum (Percent of control) ± S.E. |
|--------------|--------------------------------------------|--------------------------------------|-----------------------------------|
| DDVP         | 56.4±2.8***                               | 73.1±1.7**                           | 72.4±4.0***                       |
| DDVP+Diethyoxime | 52.7±2.5                                 | 78.8±1.6                             | 89.3±1.8                          |
| DFP          | 6.7±0.3***                                | 14.3±0.9                             | 5.3±0.4                           |
| DFP+Diethyoxime | (1)                                      | (5.8)****                            | (1.6)**                           |

DDVP/DDFP: 0.5 × LD_{50}, s.c. n=6. Diethyoxime: 50 mg/kg, i.m., 1 min after OP intoxication. *: Percentage reactivation = \( \frac{Ar-Ai}{Ac-Ai} \) × 100, where Ar=Activity after reactivation, Ai=Activity after inhibition, Ac=Activity control. **P<0.05, ***P<0.01, ****P<0.001. 1Control ChE activity: 26.55±0.64 nmols mg protein^{-1} min^{-1} in the corpus striatum, 3.87±0.09 nmols mg protein^{-1} min^{-1} in the diaphragm, 289.20±4.60 nmols ml serum^{-1} min^{-1}.

| Groups           | DDVP poisoning | DFP poisoning |
|------------------|----------------|---------------|
|                 | LD_{50} (mg/kg) | PI            | LD_{50} (mg/kg) | PI            |
| None             | 11.2±1.06      | —             | 3.3±0.11       | —             |
| Diethyoxime     | 23.6±1.10      | 2.1           | 3.3±0.11       | 1.0           |
| Atropine         | 24.3±1.07      | 2.2           | 4.5±1.05       | 1.4           |
| Atropine+Diethyoxime | 41.9±1.08     | 3.6           | 5.5±1.16       | 1.7           |

LD_{50} of diethyoxime: 872 mg/kg, i.m. DDVP/DDFP administered s.c. Atropine (10 mg/kg), i.p., after 30 sec and diethyoxime (50 mg/kg), i.m., given 1 min after poisoning.
relatively higher in the case of DDVP than DFP poisoning as evidenced from the percent ChE reactivation. This is in accordance with the protection studies (Table 2), i.e., diethyfunctionalized ChE reactivation in DFP poisoning was not sufficient to afford protection against lethality when administered along with atropine (to suppress the muscarinic effects), while it was able to protect against it in DDVP poisoning. However, diethyfunctional when administered alone did not produce significant protection against either DDVP or DFP poisoning.

Both OP compounds in positive pressure ventilated animals produced a progressively developing tetanic fade (Table 3) and bradycardia (Table 4). Therapeutic administration of diethyfunctional produced a partial recovery of NM function in DDVP poisoning without having any beneficial effect in DFP intoxication. Administration of atropine alone prevented OP induced bradycardia.

**Discussion**

Diethyfunctional is a compound of low mammalian toxicity as evidenced from its LD$_{50}$ value, and it is relatively safe in comparison to pyridine aldoximes. Though diethyfunctional was able to reanimate OP inhibited ChE peripherally, it could not afford any significant protection against either DDVP or DFP poisoning. In accordance with its high reactivating potency of DDVP inhibited ChE than DFP, diethyfunctional when administered along with atropine offered protection against DDVP poisoning, but had no beneficial effect

---

**Table 3. Effect on tetanic performance**

| S. No. | Groups               | Average neuromuscular transmission (Percent of control) ± S.E. |
|--------|----------------------|---------------------------------------------------------------|
|        |                      | 15 min     | 30 min     | 45 min     | 60 min     |
| 1.     | DDVP                 | 50.5±7.7   | 21.6±5.4   | 16.7±3.1   | 13.2±2.1   |
| 2.     | DDVP+Diethyfunctional | 80.5±2.1*  | 49.5±3.5** | 32.9±2.0** | 30.7±2.9** |
| 3.     | DDVP+Atropine        | 52.7±4.6   | 25.4±3.6   | 17.3±2.1   | 14.5±1.9   |
| 4.     | DDVP+Atropine+Diethyfunctional | 83.5±5.1 | 57.7±4.6   | 37.9±3.1   | 34.6±3.6   |
| 5.     | DFP                  | 82.4±3.6   | 44.0±6.8   | 16.8±2.8   | 2.2±0.9    |
| 6.     | DFP+Diethyfunctional | 81.7±3.5   | 45.6±4.1   | 18.6±2.7   | 2.7±0.8    |
| 7.     | DFP+Atropine         | 83.5±4.1   | 44.9±3.6   | 17.5±3.2   | 2.8±0.7    |
| 8.     | DFP+Atropine+Diethyfunctional | 82.7±5.9 | 46.5±5.9   | 19.6±3.8   | 3.0±0.8    |

*Control neuromuscular transmission: 33.72±1.41 mm, *P*<0.01 as compared to Group No. 1. **P*<0.001 as compared to Group No. 1. DDVP/DDVP: 3.5 LD$_{50}$, s.c. n=8. Atropine (10 mg/kg), i.p., after 30 sec and diethyfunctional (100 mg/kg), i.m., given 1 min after poisoning.

**Table 4. Effect on heart rate**

| S. No. | Groups               | Average heart rate (Percent of control) ± S.E. |
|--------|----------------------|------------------------------------------------|
|        |                      | 15 min     | 30 min     | 45 min     | 60 min     |
| 1.     | DDVP                 | 59.2±3.3   | 58.0±4.1   | 55.0±3.3   | 54.8±3.1   |
| 2.     | DDVP+Diethyfunctional | 61.8±3.6   | 59.9±3.9   | 58.8±4.1   | 56.3±3.2   |
| 3.     | DDVP+Atropine        | 92.1±2.8*  | 90.5±3.4*  | 82.3±6.4*  | 82.4±2.9*  |
| 4.     | DDVP+Atropine+Diethyfunctional | 93.0±2.2 | 89.3±3.5   | 83.3±3.7   | 82.1±4.2   |
| 5.     | DFP                  | 95.0±5.5   | 37.0±3.1   | 28.8±3.8   | 19.1±3.1   |
| 6.     | DFP+Diethyfunctional | 94.2±4.6   | 39.2±3.2   | 30.5±4.1   | 21.2±3.7   |
| 7.     | DFP+Atropine         | 98.1±2.6   | 93.4±2.5** | 86.4±3.7** | 84.2±4.6** |
| 8.     | DFP+Atropine+Diethyfunctional | 98.0±5.0 | 95.6±1.6   | 87.6±2.9   | 88.2±2.7   |

*Control heart rate: 392.0±7.7/min. *P*<0.001 as compared to Group No. 1. **P*<0.001 as compared to Group No. 5. DDVP/DDVP: 3.5 LD$_{50}$, s.c. n=8. Atropine (10 mg/kg), i.p., after 30 sec and diethyfunctional (100 mg/kg), i.m., given 1 min after poisoning.
in DFP intoxication. It may be due to either prolonged persistence of DFP in the biosystem (18) or rapid ageing of DFP inhibited ChE as compared to DDVP. Although diethyxime has been claimed to possess a central ChE reactivating property (5), our study indicated its inability to reactivate ChE enzyme in the central nervous system during DDVP as well as in DFP poisoning as evidenced by no reactivation in the corpus striatum.

The observed DDVP and DFP induced bradycardia may be due to a peripheral muscarinic effect (19). This was counteracted by the administration of a cholinolytic, viz. atropine. The administration of diethyxime could not produce further beneficial effect on heart rate during OP intoxication. Experimental evidence indicates that OP induced NM blockade is due to prolonged depolarization on the muscle end plate (20). Administration of diethyxime produced a partial recovery of DDVP induced NM blockade. However, this regimen could not prevent the deleterious effect of DFP on NM function.

Based on the effect of diethyxime on different parameters, it may be concluded that it is an effective peripheral reactivator against a less potent anticholinesterase (anti-ChE) agent like DDVP and without any antidotal efficacy against a more potent anti-ChE agent like DFP. It is seemed that diethyxime may not be a universal antidote against OP compounds intoxication as claimed earlier by Kokshareva et al. (5); however, it may be of use as an antidote against low toxic OP insecticides.

Acknowledgements: The authors thank Dr. P.K. Ramachandran, Director, Defence Research and Development Establishment Gwalior for his keen interest in this work. Thanks are also due to Mr. Hari Afley for secretarial assistance.

References
1 Vojvodic, V. and Boskovic, B.: Comparative study of pralidoxime, obidoxime and trimedoxime in healthy men volunteers and in rats. In Medical Protection against Chemical-Warfare Agents, Stockholm International Peace Research Institute, p. 65–73. Almqvist and Wiskell, Stockholm (1976)
2 Ellin, R.I. and Wills, J.H.: Oximes antagonistic to inhibitors of cholinesterase. J. Pharm. Sci. 53, 995–1007 (1964)
3 Kenley, R.A., Howd, R.A., Mosher, C.W. and Winterle, J.S.: Non-quaternary cholinesterase reactivators: Dialkylaminoalkyl thioesters of α-ketothiohydroximic acids as reactivators of di-isoproplyphosphorofluoridate inhibited acetylcholinesterase. J. Med. Chem. 24, 1124–1133 (1981)
4 Benschop, H.P., Vandenberg, G.R., Van Hoooidonk, C., Dejong, L.P.A., Kientz, C.E., Berends, F., Kepner, L.A., Meeter, E. and Visser, R.P.L.S.: Antidotes to organophosphate poisoning. 2. Thiaziazole-5-carboxidoximes. J. Med. Chem. 22, 1306–1313 (1979)
5 Kokshareva, N.V., Kotvum, S.D., Kagan, Yu.S., Mizyukova, I.G. and Medvedev, B.M.: Effect of a new cholinesterase reactivator, diethyxime, on the central nervous system. Byull. Eksp. Biol. Med. 83, 29–32 (1977) (Abs. in English)
6 Das Gupta, S., Ghosh, A.K., Moorthy, M.V., Jaiswal, D.K., Chowdhri, B.L., Purnanand and Pant, B.P.: Comparative studies of pralidoxime, trimedoxime, obidoxime and diethyxime in acute fluorosigmine poisoning in rats. Pharmazie 37, 605 (1983)
7 Cherbuliez, E.: Organic derivatives (esters and organic anhydro acids) of phosphoric and polyphosphoric acids. In Organic Phosphorus Compounds. Edited by Kosolapoff, G.M. and Maier, L., Vol. 6, p. 17–578, Wiley Interscience, Inc., New York and London (1973)
8 Krivenchuk, V.E. and Petrun'kin, V.E.: Thiohydroximic esters. I. S-Diethylaminoethyl esters of thiohydroximic acids, and their derivatives. Khim-Farm. Zh. 7, 13–16 (1973) (in Russian)
9 Pant, B.P. and Jaiswal, D.K.: Thiohydroximic s-esters as reactivators of organophosphorus inhibited cholinesterase. Ind. J. Chem. 22B, 51–53 (1983)
10 Dixon, W.J.: The up-and-down method for small samples. J. Am. Statist. Assoc. 60, 967–978 (1965)
11 Glowinski, J. and Iversen, L.L.: Regional studies of catecholamines in the rat brain. I. The disposition of 3H-norepinephrine, 3H-dopamine and 3H-DOPA in various regions of rat brain. J. Neurochem. 13, 655–669 (1966)
12 Sterri, S.H., Rognerud, B., Fiskum, S.E. and Lyngass, S.: Effect of toxogonin on PS2 on the toxicity of carbamates and organophosphorus compounds. Acta Pharmacol. Toxicol. 45, 9–15 (1979)
13 Lancaster, R.: Inhibition of acetylcholinesterase in the brain and diaphragm of rats by a tertiary organophosphorus anticholinesterase and its
quaternary analogue: in vivo and in vitro studies. J. Neurochem. 19, 2587–2597 (1972)

14 Ellman, G.L., Courtney, K.D., Andres, V., Jr. and Featherstone, R.M.: A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7, 88–95 (1961)

15 Gornall, A.G., Bardawill, C.J. and David, M.M.: Determination of serum proteins by means of the biuret reaction. J. Biol. Chem. 177, 761–766 (1948)

16 Wolthius, O.L., Berends, F. and Meeter, E.: Problems in the therapy of soman poisoning. Fundam. Appl. Toxicol. 1, 183–192 (1981)

17 Van Halden, H.P.M., Vander Wiel, H.J. and Wolthius, O.L.: Therapy of organophosphorus poisoning: the marmoset as a model for man. Br. J. Pharmacol. 78, 579–589 (1983)

18 Reynolds, J.E.F.: Neostigmine and other parasympathomimetics. In Martindale, The Extra Pharmacopoeia, p. 1039–1046, The Pharmaceutical Press, London (1982)

19 Heymans, C., Pochet, A. and Van Houtte, H.: Contributions à la pharmacologie du sarin et du tabun, Arch. Int. Pharmacodyn. Ther. 104, 293–332 (1956)

20 The sel, S. and Qu astel, D.M.J.: Neuromuscular pharmacology. Annu. Rev. Pharmacol. 5, 263–284 (1965)