Arh hig rada toksikol 1990;41:19 – 24

Communication
UDC 615.099.08

THE EFFECT OF PYRIDOSTIGMINE AND PHYSOSTIGMINE ON ACUTE TOXICITY OF DIISOPROPYL FLUOROPHOSPHATE IN RATS

K. Husain, R. Vijayaraghavan and D. N. Marjit

Division of Pharmacology, Toxicology and Chemistry, Defence Research and Development Establishment, Gwalior, India

Received December 21, 1988.

Diisopropyl fluorophosphate (DFP) given to rats in lethal concentration (100 mg/m^3, by inhalation for 40 min) significantly inhibited acetylcholinesterase activity in the blood, lung, liver and brain, and induced hyperglycaemia and glycogen mobilization in the liver, diaphragm and brain. Pretreatment (maximum sign-free dose) with carbamates, pyridostigmine (0.075 mg/kg, i.m.) or physostigmine (0.1 mg/kg, i.m.) 15 min before exposure to DFP, modified the inhibited acetylcholinesterase activity only in peripheral tissues. However, the hyperglycaemia and glycogen depletion induced by DFP inhalation were not modified by carbamate pretreatment. The time of survival of DFP exposed animals increased after pretreatment with carbamates, more after physostigmine (81 min) than after pyridostigmine (59 min). The animals exposed to DFP exhibited severe tremors and convulsions as compared to the animals pretreated with carbamates.

DFP (diisopropyl fluorophosphate), an irreversible organophosphorus cholinesterase inhibitor is known to produce acute toxic effects such as hyperactivity, tremor, convulsion, salivation, respiratory impairment, hyperglycaemia and glycogenolysis in humans and various species of animals (1, 2). Treatment of organophosphorus toxicity is usually by the cholinolytic atropine combined with a cholinesterase reactivator and the anticonvulsant diazepam (3, 4). Pretreatment with carbamates, like pyridostigmine and physostigmine, was found to be beneficial against organophosphorus toxicity (5). *Koster* (6) was the first to report that physostigmine pretreatment offers considerable protection against DFP in cats. Work from our laboratory (7) confirmed the beneficial effect of pyridostigmine and physostigmine against acute DFP intoxication in rats. The rationale for this protection is due to carbamylation of cholinesterase to render it temporarily insensitive to irreversible inhibition by organophosphates. Reports on the

protective effect of carbamates against DFP poisoning by inhalation are scanty. Since the major route of entry of DFP is by inhalation, the present study was undertaken to investigate the effect of carbamate pretreatment against the acute toxic effects of DFP acrosols in rats.

MATERIAL AND METHODS

Adult male albino rats weighing 110 – 130 g bred in our establishment were used in experiment. They were housed in polypropylene cages, four per cage. Food (Lipton feed) and water were freely available except before exposure when the animals were fasted overnight. DFP was synthetized in our chemistry division and was found to be 99% pure by gas chromatographic analysis. Pyridostigmine (3-hydroxy-1-methyl pyridinium dimethyl carbamate) and physostigmine (1,2,3,3aβ,8,8aβ-hexahydro--1,3a,8-trimethyl pyrrolo[2,3-b]-indol-5 yl methyl carbamate) were obtained from Sigma Chemicals Co. St.Louis, U.S.A. For exposure to DFP by inhalation a dynamically--operated all-glass whole body chamber was used. Rats, three at a time, were exposed in stainless steel wire cages, one per cage. DFP aerosols were generated by a nebulising dilute solution of DFP in distilled water and the solution was fed into the liquid pick-up tube of an air blast nebulizer through a syringe infusion pump. The concentration was determined by gas liquid chromatography. Analysis of particles by size was carried out with a Royco Particle Monitor. Ninety-nine per cent of the particles were within the respirable range (< 0.3 μm). A maximum sign-free dose (5, 8) of pyridostigmine (0.075 mg/kg, i.m.) or physostigmine (0.1 mg/kg, i.m.) was injected to rats 15 min before exposure. The rats were exposed to DFP aerosols (100 mg/m^3) for 40 min, and killed immediately by microwave irradiation (0.6 mw/cm^2 for 2 sec). Blood was collected from the heart in heparinized tubes and used for the estimation of blood glucose by the method of *Nelson* (9). The liver, lung, diaphragm and brain were quickly removed after dissection and weighed. Glycogen was extracted from the liver, diaphragm and brain by the method of *LeBaron* (10) and estimated as described by *Montgomery* (11). For the assay of acetylcholinesterase (EC. 3.1.1.7) activity, blood cells were lysed with saponin and diluted (1:200) with phosphate buffer pH 8.0. The tissues were homogenized in phosphate buffer pH 8.0 to give a final concentration of 20 mg/ml, w/v, and activity was assayed spectrophotometrically as described by *Ellman and co-workers* (12) using acetylthiocholine as substrate.

Blood cholinesterase activity was also determined at 15 min intervals up to 120 min in separate groups of rats administered pyridostigmine and physostigmine (maximum sign-free dose). In another set of experiments rats were pretreated with a maximum sign-free dose of pyridostigmine or physostigmine 15 min before being exposed to DFP aerosols (100 mg/m^3) until death. The data were analysed statistically by Student's t-test.

RESULTS

The results presented in Table 1 show that DFP significantly inhibited acetylcholinesterase activity in the blood, brain, lung and liver of rats. The inhibition of enzyme activity was more pronounced in the blood and brain than in the liver and lung. In pyridostigmine and physostigmine pretreated rats the enzyme activity was significantly higher only in the peripheral tissues. The survival time for animals exposed only to DFP was 40 min, whereas for those pretreated with pyridostigmine and physostigmine it was 59 and 81 min respectively. The results summarized in Table 2 indicate that DFP significantly increased the level of blood glucose and depleted glycogen level in the brain, liver and diaphragm. Pretreatment with pyridostigmine or physostigmine produced no major effect on hyperglycaemia and glycogenolysis induced by DFP inhalation in rats. The animals exposed to DFP exhibited severe tremors and convulsions, whereas those pretreated with pyridostigmine or physostigmine showed mild convulsions and tremors.

Table 1.

Effect of pyridostigmine (0.075 mg/kg, i.m.) and physostigmine (0.1 mg/kg, i.m.) pretreatment on acetylcholinesterase activity in cerebral and peripheral tissues and survival time of rats exposed to DFP (100 mg/m³).

Groups	Acetylcholinesterase* (μmoles acetylthiocholine hydrolyzed/min/g tissue, or ml blood)				Survival time** (min)
	Brain	Blood	Liver	Lung	
1. Control	5.15 ± 0.24	3.02 ± 0.12	1.48 ± 0.06	1.22 ± 0.04	–
2. DFP	0.88 ± [a] 0.03	0.43 ± [a] 0.03	0.42 ± [a] 0.02	0.34 ± [a] 0.02	39.6 ± 5.1
3. Py + DFP	0.96 ± [d] 0.07	0.75 ± [b] 0.05	0.55 ± [c] 0.04	0.42 ± [b] 0.01	58.6 ± [b] 7.6
4. Ph + DFP	0.90 ± [d] 0.05	0.73 ± [c] 0.10	0.65 ± [b] 0.03	0.52 ± [c] 0.06	81.3 ± [b] 4.5

Pyridostigmine (Py) and physostigmine (Ph) were given 15 min prior to DFP inhalation.

Significantly different from the values: [a] of Group 1 ($P < 0.001$), [b] of Group 2 ($P < 0.001$), [c] of Group 2 ($P < 0.01$), [d] not significant as compared to Group 2.

* DFP exposure for 40 min, mean ± SE of six animals.

** Mean ± SE of ten animals.

Table 2.

Effect of pyridostigmine (0.075 mg/kg, i.m.) and physostigmine (0.1 mg/kg, i.m.) pretreatment on the level of blood glucose and glycogen in cerebral and peripheral tissues in DFP (100 mg/m³) exposed rats. Animals were killed 40 min after DFP inhalation. Each figure represents mean ± SE of six animals

Groups	Blood glucose (mg/100 ml)	Glycogen (mg/100 g tissue)		
		Brain	Liver	Diaphragm
1. Control	90.04 ± 4.60	90.50 ± 5.60	503.48 ± 12.62	148.68 ± 6.81
2. DFP	169.92 ± [a] 5.90	50.85 ± [a] 4.58	318.99 ± [a] 9.63	93.66 ± [a] 4.59
3. Py + DFP	180.61 ± [b] 4.56	51.08 ± [b] 3.99	298.98 ± [b] 7.59	100.40 ± [b] 3.58
4. Ph + DFP	173.79 ± [b] 7.02	56.80 ± [b] 3.81	315.44 ± [b] 8.08	101.66 ± [b] 3.49

Pyridostigmine (Py) and physostigmine (Ph) were injected 15 min before DFP exposure.

[a] Significantly different from values of Group 1 ($P < 0.001$)
[b] Not significant as compared to Group 2.

DISCUSSION

It is well known that acute toxic effects induced by organophosphates and carbamates are mainly due to inhibition of acetylcholinesterase in nervous tissues (1, 2). While organophosphorus compounds phosphorylate the active site of cholinesterases more or less irreversibly, depending on their chemical structure, carbamates carbamylate the enzyme site in a fully reversible manner (13, 14). In our present study DFP significantly inhibited the acetylcholinesterase of cerebral and peripheral tissues (Table 1), but in animals pretreated with pyridostigmine or physostigmine acetylcholinesterase was somewhat protected as shown by its higher activity in peripheral tissues and by the increased survival time. It is known that physostigmine can easily cross the blood-brain barrier, whereas pyridostigmine, which has quarternary nitrogen in its structure, cannot (15). Organophosphorus compounds, if inhaled, do not only interact with pulmonary acetylcholinesterase but can also inhibit blood and extrapulmonary acetylcholinesterases (16). In our study the maximal inhibition of enzyme activity was observed in the blood and brain and the minimal one in the liver and lungs, which is in agreement with the reported findings. Pretreatment with pyridostigmine or physostigmine neither abolished the hyperglycaemia nor modified glycogenolysis induced by DFP in cerebral and peripheral tissues of animals.

Albuquerque and co-workers (17) reported that pretreatment with physostigmine and atropine modified brain acetylcholinesterase inhibition induced by low doses of sarin and that the degree of protection was the same if the dose of sarin was increased. In our observation carbamate pretreatment reduced the severity of tremors and convulsions induced by DFP in rats. Although brain acetylcholinesterase was not significantly protected by physostigmine, the protection of acetylcholinesterase in peripheral tissues following physostigmine and pyridostigmine pretreatment was more or less the same. The time of survival for rats pretreated with physostigmine was significantly longer than for rats pretreated with pyridostigmine. The interaction of carbamates with the peripheral nicotine acetylcholine receptor – ionic channel may be responsible for this protection (17, 18). Other factors, such as rates of absorption, distribution, metabolism and excretion, will also determine the effectiveness of carbamates in protecting animals against organophosphate poisoning.

Acknowledgements – The authors are grateful to Brig. K.M. Rao, Director, Dr. P.K. Ramachandran, Scientist Emeritus and Dr. S. Das Gupta, Head, Division of Pharmacology and Toxicology, Defence Research and Development Establishment, Gwalior for their keen interest and encouragement in this study.

REFERENCES

1. *Holmstedt B.* Pharmacology of organophosphorus cholinesterase inhibitors. Pharmacol Rev 1959;11:569–688.
2. *Namba T, Jackvel GT, Grob D.* Poisoning due to organophosphate insecticides. Am J Med 1971;50:475–92.
3. *Matin MA, Husain K.* The effect of certain drugs on glycogen and acetylcholine levels in cerebral and peripheral tissues in rats with malathion induced hyperglycaemia. Arch Ind Hyg Toxicol 1984;35:325–332.
4. *Johnson DD, Wilcox WC.* Studies on the mechanism of the protective and antidotal actions of diazepam in organophosphate poisoning. Eur J Pharmacol 1975;34:127–32.
5. *Berry WK, Davies DR.* The use of carbamates and atropine in the protection of animals against poisoning by 1,2,2-trimethyl propyl methyl phosphofluoridate. Biochem Pharmacol 1970;927–34.
6. *Koster R.* Synergism and antagonism between physostigmine and diisopropylfluorophosphate in cats. J Pharmacol Exp Ther 1946;88:39–46.
7. *Das Gupta S, Ghosh AK, Jeevarathinam K.* Beneficial effect of carbamates against fluostigmine poisoning in rats. Die Pharmazie 1987;42:206–7.
8. *Dirnhuber P, French MC, Green DM, Leadbetter L, Stratton A.* The protection of primates against soman poisoning by treatment with o pyridostigmine. J Pharm Pharmacol 1979;295–9.
9. *Nelson N.* A photometric adaptation of the Somogyi method for the determination of glucose. J Biol Chem 1944;153:375–80.
10. *LeBaron FN.* The resynthesis of glycogen by guinea pig cerebral cortex slices. Biochem J 1955;61:80–5.
11. *Montgomery R.* Determination of glycogen. Arch Biochem Biophys 1957;67:378–86.
12. *Ellman GL, Courtney KD, Andre V Jr, Featherstone RM.* A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961;7:88–95.

13. *O'Brien RD, Hetnarski B, Tripathi RK, Hart GJ.* Recent studies of acetylcholinesterase inhibition. In: G.K. Kohn, ed. Mechanism of pesticide action. Washington: American Chemical Society, 1974:1 – 13.
14. *Wilson IB, Harrison MA, Ginsburg S.* Carbamyl derivaties of acetylcholinesterase. J Biol Chem 1961;236:1498 – 500.
15. *Birtley RDN, Roberts JB, Thomas BH, Wilson A.* Excretion and metabolism of ^{14}C-Pyridostigmine in the rat. Br J Pharmacol 1966;26:393 – 402.
16. *Pauluhn J, Machemer L, Kimmerle G.* Effects of inhaled cholinesterase inhibitors on bronchial tonus and on plasma and erythrocyte acetylcholinesterase activity in rats. Toxicology 1987;46:177 – 90.
17. *Albuquerque EX. et al.* Multiple actions of anticholinesterase agents on chemosensitive synapses: Molecular basis for prophylaxis and treatment of organophosphate poisoning. Fundam Appl Toxicol 1985;5:5182 – 203.
18. *Akaike A, Ikeda SR, Brookes N, Pascuzzo GJ, Rickett OL, Albuquerque EX.* The nature of interactions of pyridostigmine with the nicotine acetylcholine receptor – ionic channel complex. II-Patch clamp studies. Mol Pharmacol 1984;25:102 – 12.

Sažetak

UČINAK PIRIDOSTIGMINA I FIZOSTIGMINA NA AKUTNU TOKSIČNOST DIIZOPROPIL FLUOROFOSFATA U ŠTAKORA

Diizopropil fluorofosfat (DFP), koji su štakori primili u letalnoj koncentraciji (100 mg/m^3) inhalacijom tijekom 40 minuta, značajno je inhibirao aktivnost acetilkolinesteraze u krvi, plućima, jetri i mozgu te izazvao hiperglikemiju i doveo do mobilizacije glikogena u jetri, dijafragmi i mozgu. Prethodno intramuskularno tretiranje životinja maksimalnom dozom karbamata koja još ne izaziva znakove trovanja, i to piridostigminom (0,075 mg/kg) ili fizostigminom (0,1 mg/kg) petnaest minuta prije ekspozicije DFP-u dovelo je do promjene u inhibiciji aktivnosti acetilkolinesteraze samo u perifernim tkivima. Međutim, na hiperglikemiju i nedostatnost glikogena koji su bili izazvani inhaliranjem DFP-a prethodno tretiranje karbamatima nije utjecalo. Vrijeme preživljenja životinja koje su bile izložene DFP-u bilo je duže nakon što su životinje primile karbamate. U tom pogledu fizostigmin (81 min) je bio djelotvorniji od piridostigmina (59 minuta). U životinja koje su bile izložene DFP-u primijećeni su jači tremor i konvulzije nego u životinja koje su ranije bile tretirane karbamatima.

Odjel za farmakologiju, toksikologiju i kemiju, Ministarstvo za obranu, istraživanje i razvoj, Gwalior, Indija