Bull. Environ. Contam. Toxicol. (1997) 59:246-253

Bulletin of Environmental Contamination and Toxicology

Cardiorespiratory and Neuromuscular Effects of O-Ethyl S-[2-(Diisopropylamino) Ethyl] Methylphosphonothioate (VX) in Rats

S. N. Dube,[1] P. K. Mazumder,[1] D. Kumar,[1] Pumanand [2]

[1]Divisions of Pharmacology and Toxicology, and [2]Synthetic Chemistry Defence Research and Development Establishment, Jhansi Road, Gwalior 474 002, India

Received: 6 January 1997/Accepted: 14 May 1997

The exposure to chemical warfare agents is not restricted to the battlefield but as humans may be poisoned with nerve gases either from a chemical warfare stockpile or even in a public place. The recent use of nerve gas Sarin in a subway of Japan by a fanatic group has triggered global consternation about the extremists and homemade weapons of mass destruction. Nerve agents are organophosphorus (OP) compounds that exert acute toxic manifestations principally by inhibiting the enzyme acetylcholinesterase (AChE) resulting in a variety of physiological alterations and ultimately death from respiratory failure (Brimblecombe 1977). However, pharmacological studies indicate that nerve agents have plethora of actions other than inhibition of AChE (Worek and Szinicz 1995, Corbier and Robineau 1989, Robineau and Guittin 1987).

Several studies have revealed that acute intravenous (i.v.) administration of an OP agent increased blood pressure in cat, dog and rat (Brezenoff and Giuliano 1982, Lang and Rush 1973), while subcutaneous (s.c.) administration produced marked hypotension (Preston and Heath 1972). In a recent communication from this laboratory, it has been reported that i.v. administration of DFP/sarin produced a dose-dependent hypertension involving cholinergic-catecholaminergic interaction in rats. Subcutaneous administration of DFP/ sarin was found to produce a dose-responsive hypotension mediated through muscarinic receptors (Dube *et al* 1993). However, the mechanism of cardiovascular toxicity induced by nerve agents still remains obscure. Therefore, the effects on blood pressure, heart rate, tracheobronchial response, neuromuscular transmission, and modulatory role of various pharmacological agents were studied after systemic administration of VX (O-ethyl S-[2- (diisopropylamino) ethyl] methyl phosphonothioate) to delineate the distinction in mechanism of action from other OP compounds.

Correspondence to: S. N. Dube

MATERIALS AND METHODS

Male Wistar rats (150-200g) were bred in Animal Facility of Defence R & D Establishment, Gwalior and were housed in propylene cages and provided gold mohur laboratory feed from Brooke Bond Lipton India Limited, India and water *ad libitum.*

The nerve agent, VX was synthesized in the Synthetic Chemistry Division of Defence R & D Establishment, Gwalior. O-Ethyl methylphosphonothioic acid on reaction with N,N-diisopropylethyl chloride in the presence of triethylamine yields VX with >95% purity as confirmed by gas chromatographic analysis. Atropine sulphate was purchased from Plantex Ltd, Israel. Tolazoline and all other drugs/ chemicals were procured from Sigma Chemical Co.(St. Louis, MO, USA) and RBI USA.

Rats were anaesthetised with pentobarbitone sodium (40 mg/kg,i.p.) for recording various physiological parameters on a Grass Polygraph (Model 7-16P-35). In order to eliminate the influence of vagus activities especially on the cardiovascular system, bilateral vagotomy was performed and the animals were maintained on positive pressure ventilation using a rodent ventilator (Ugo Basile Model 7025, Comerio, Italy) whenever required. A bronchospasm transducer (Ugo Basile Model 7020, Italy) was used to measure the tracheobronchial constriction/spasm; i.e. tracheobronchial response (TBR). The signals generated were fed to a low level D.C. preamplifier (Model 7Pl) to record the TBR. The carotid artery was cannulated and connected to a Statham transducer to record blood pressure (BP) using a low level D.C. preamplifier (Model 7Pl). The pulse signals were also fed into the tachograph preamplifier (Type 7P4) to record heart rate (HR). Neuromuscular transmission (NMT) of the gastrocnemius muscle was recorded using force transducer (Model FT 03) as described earlier (Dube *et al* 1993).

Bilateral adrenalectomy was performed under ether anaesthesia and both adrenal glands were removed aseptically as described by Matin et al (1989). Antibiotic administration was stopped 48 hrs before the actual experiment and the adrenalectomised rats were used on 10th day following surgery.

The physiological responses (BP, HR, TBR and NMT) were studied after a stabilization period of about 30-60 min. The same variables were also recorded following systemic administratation (i.v. and s.c.) of various doses of VX (5, 10, 20 and 40 µg/kg) at 15 min interval. Each animal received only one dose of VX, whereas control animals received an equal volume of normal saline and maintained similar to experimental animals. Each group consisted of 5 rats.

Data are expressed as means ± S.E. and are presented as percent of initial basal control values. Statistical significance was taken as $P<0.05$ by student's 't' test.

RESULTS AND DISCUSSION

The absolute basal values varied between 332 ± 22 and 384 ± 23 (heart beat/min); 92±8 and 114 ± 10 mm Hg (mean atrerial pressure). The i.v. administration of VX (5, 10, and 20 µg/kg) in positive-pressure ventilated rats produced a dose-dependent prolonged increase in blood pressure (106-130 % of control), a decrease in heart rate (94-57% of control) and potentiation of the simple muscle twitch (102-130 % of control) at 2-30 min (Figure 1 and , Table 1) without showing any significant effect on tracheo bronchial response (data not shown). A higher dose of VX (40 µg/kg,i.v.) produced a marked rise in blood pressure (160% of control) and a decrease in heart rate (43 % of control) leading to shock and ultimately death within 15-30 min (Table 1).

The administration of VX by a s.c.route produced a dose-dependent transient decrease in blood pressure (85-68 % of control) at 2 min with a tendency towards recovery after 30 min (Table 1). However, the decrease in heart rate was dose responsive and maintained even after 30 min, in spite of the normal blood pressure. There was no significant effect on simple muscle twitch and tracheobronchial response 2-30 min following s.c. administration of various doses of VX.

Bilateral adrenalectomy as well as tolazoline pretreatment (5 mg/kg, i.v.) did not modify the cardiovascular response to intravenously administered VX. The vasopressor and bradycardiac responses also remained unaltered (data not shown) with pretreatments of hexamethonium (1 mg/kg) and propranolol (1 mg/kg). However, the pretreatment with atropine (10 mg/kg, i.v.) completely antagonised the hypertensive and bradycardiac effect of intravenously administered VX (Figure 2). Pretreatment with atropine also antagonized the transient hypotension, bradycardia, and potentiation of muscle twitch following subcutaneous administration of VX.

The toxic manifestations during OP intoxication result from inhibition of AChE leading to an accumulation of acetylcholine in synaptic junctions. Death due to OP intoxication attributed to respiratory failure (Willette *et al* 1984). However, recent studies have demonstrated that the toxicological action on cardiac control mechanisms is equally important in producing lethality (Takahashi *et al* 1991). Several reports indicate that in most species the predominant effect of a systemic intoxication with OP is a fall in blood pressure and bradycardia (Robineau and Guittin 1987, Worek *et*

al 1995a and 1995b). VX administration by i.v. route can cause a rapid and severe bradycardia and an initial increase in blood pressure followed by a slow but progrssive hypotension in anaesthetized guinea-pigs (Worek and Szinicz 1995). However, in contrast to this we observed a prolonged increase in blood pressuire following i.v. administration of VX in rats, suggesting that there is a species dependent effect of VX on blood pressure. The vasopressor response and bradycardia induced by i.v. VX adminstration as observed in the present study in rats is in agreement with earlier reports in cat, dog, and rat (Brezenoff and Giuliano 1982).

Table 1 Effect of VX administration on blood pressure (BP), heart rate (HR) and neuromuscular transmission (NMT) in anaesthetised and artificially ventilated rats. Values are Mean % control ± SEM, n=5; a=P<0.05 as compared to control.Tolazoline 5 mg/kg, i.v. 30 min before; Atropine sulphate 10 mg/kg, i.v.10 min before.

Dose of VX	Route	BP		HR		NMT	
µg/kg		2 min	30 min	2 min	30 min	2 min	30 min
5	i.v.	105±5	112±5	94±3	89±4a	101.5±2.1	102.5±3.5
10	i.v.	113±4a	119±6	88±3a	80±3a	110.2±3.1a	116.5±2.1a
20	i.v.	130±6a	139±6a	170±3a	57±2a	115.7±4.5a	130.2±3.6a
10	s.c.	85±3a	97±4	97±4	88±3a	102.5±2.8	104.2±3.7
20	s.c	77±3a	92±3a	89±4a	81±3a	103.2±3.5	103.7±2.5
40	s.c.	68±3a	91±4a	83±3a	76±2a	101.7±4.7	105.2±3.8
After adrenalectomy							
10	i.v	115±4a	120±4a	91±5	82±4a	108.7±2.5	117.5±3.7a
20	i.v.	129±5	140±5a	72±3a	58±3a	116.3±3.9a	128.5±4.6a
20	s.c.	76±4	92±4a	89±4a	81±4a	104.2±3.7	105.5±5.6
40	s.c.	70±4a	89±3a	82±4a	79±4a	102.7±4.7	107.2±5.1
After tolazoline pretreatment							
10	i.v.	110±3a	118±5a	91±4a	83±2a	108.2±3.9	114.5±4.1a
20	i.v.	127±6a	136±5a	72±4a	60±2a	113.7±4.8a	127.2±5.6a
After atropine pretreatment							
10	i.v.	103±2	105±5	98±2	98±3	102.7±3.5	101.2±3.2
20	i.v.	106±4	108±6	96±4	98±3	106.1±4.5	107.2±5.6
20	s.c.	102±3	104±4	97±2	100±3	103.3±3.8	105.2±6.7
40	s.c.	106±4	105±3	98±3	97±3	102.6±3.7	106.3±4.7

The s.c. administration of VX produced a dose dependent hypotension and bradycardia. Our results are in agreement with the work of Robineau and Guittin (1987) where they observed a significant decrease in heart rate, atrial and left intraventricular pressures, contractility index, lengthening of QT interval, ventricular tachycardia and atrioventricular blocks probably

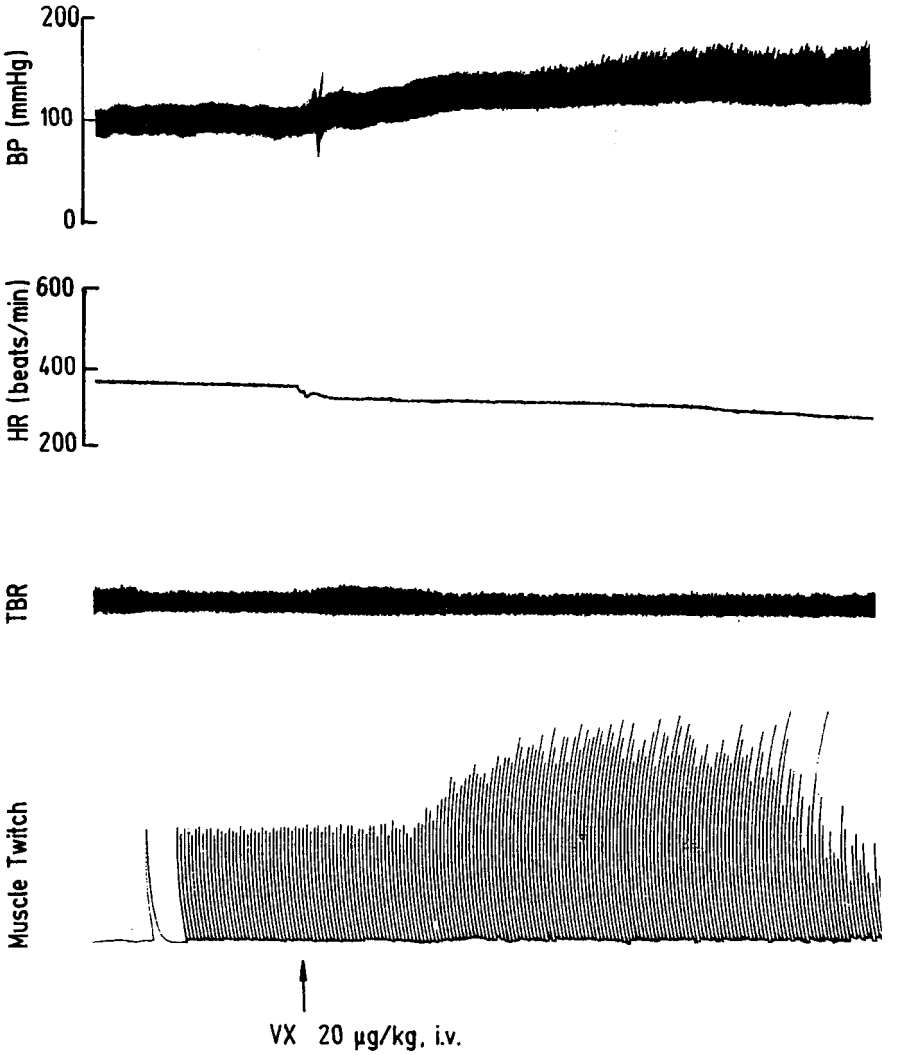

Figure 1 Effect of VX (20 µg/kg, i.v.) on blood pressure (BP), heart rate (HR), tracheobronchial response (TBR) and muscle twitch in rats.

independent of muscarinic stimulation following s.c. administration of VX in beagle dogs. Further, VX has also been shown to induce ventricular arrhythmias due to Na, K ATPase inhibition in guineapig papillary muscles (Corbier and Robineau 1989). Although atropine has been reported to aggravate the OP induced arrhythmias or to induce ventricular tachyarrhythmias or fibrillation (Baskin and Whitmer 1992) but in the present study a complete antagonism of VX induced effects on blood pressure and heart rate was observed without any deleterious effect.

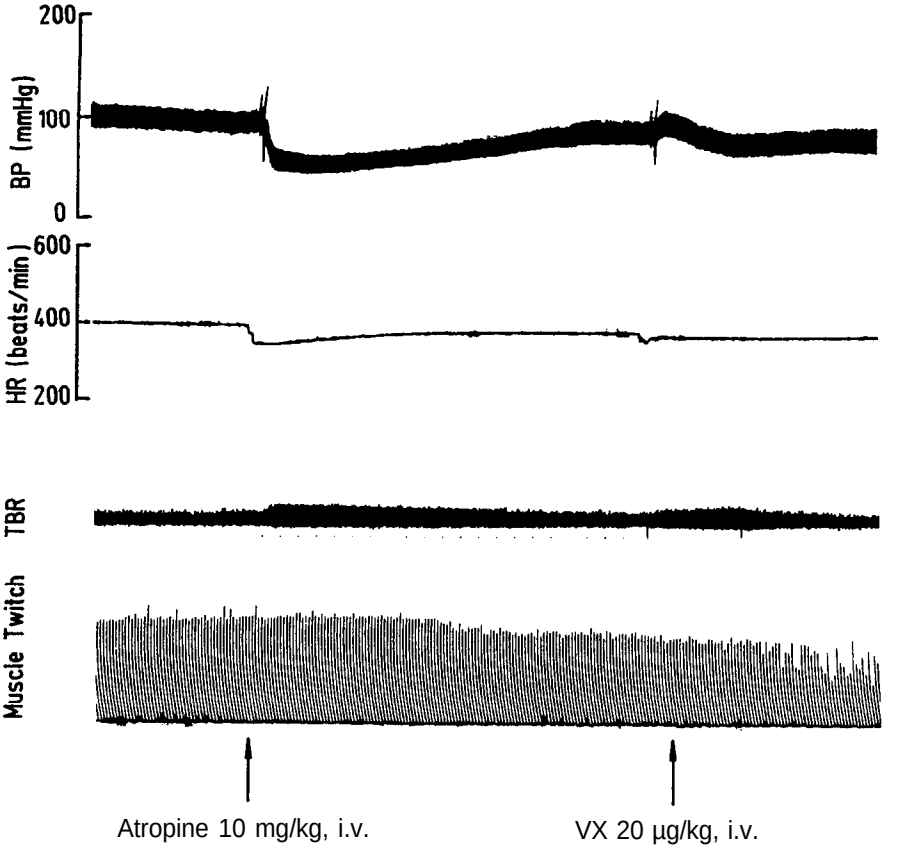

Figure 2 Effect of VX (20 µg/kg, i.v.) on blood pressure (BP), heart rate (HR), tracheobronchial response (TBR) and muscle twitch in atropine pretreated rats.

An increase in endogenous brain ACh levels at central neuronal synapses evoke a pressor response which is antagonized by atropine (Domino and Wilson 1972) or by depletion of brain ACh with hemicholinium (HC-3), which inhibits ACh synthesis (Yamamura and Snyder 1973). In the present study atropine pretreatment completely abolished VX-induced pressor responses suggesting that the hypertensive effect of VX is mediated through muscarinic receptors. This is in contrast to earlier observation where DFP/sarin-induced vasodepressor effects were antagonized by a combination of atropine and tolazoline, indicating a cholinergic-catecholaminergic interaction rather than muscarinic effect (Dube *et al* 1993). It is also reported that cholinergically evoked impulses originating in the brain produced cardiovascular responses via increased peripheral sympathetic activity (Bartholini and Pletscher 1971). Various workers have

evaluated the effects of drugs known to impair the function of sympathetic nervous system to study OP-induced, centrally mediated, cardiovascular responses (Brezenoff and Ginliano 1982). But ineffectiveness of the ganglion blocker hexamethonium, β adrenoceptor blocker propranolol and, adrenalectomy repudiates the involvement of the sympatho-adrenal system. Hypertension observed in bilateral vagotomized animals suggested a possible effect on other afferent pathways to the brain stem. This indicates that the pressor response results from a direct action on the CNS and not from a reflex increase in cardiovascular activity.

Pretreatment with atropine significantly abolished a dose- responsive hypotension and bradycardia following s.c. administration of VX. This may be attributed to the stimulation of cholinergic vasodilatory fibres sensitive to atropine. The difference in cardiorespiratory responses following two routes of challenge, with VX may be due to variation in the rate of absorption. The intravenous route provides direct access to the blood stream and large vascular surface areas for absorption. Whereas the subcutaneous route leads to slower absorption because of the limited access to vascular beds (Franz and Hilaski 1990).

The results from this study indicate that the effect of VX on cardiovascular system is species and route specific in anaesthetized rats and these effects are mediated through muscarinic receptors. it seems logical to conclude that the cholinergic system is predominant in mediating the cardiovascular toxicity of VX which needs further investigation.

Acknowledgements

Authors thank Dr. R.V. Swamy, Director and Dr. R. Vijayaraghavan, Head of the Division of Pharmacology & Toxicology, Defence Research and Development Establishment, Gwalior for their keen interest in the study.

REFERENCES

Baskin SI, Whitmer MP (1992) Cardiac effects of anticholinesterase agents. In: Ballantyner B and Marrs TC (Eds.) Clinical and Experimental Toxicology of Organophosphates and Carbamates, pp 135-144, Butterworth and Heinemann, Oxford.

Bartholini G, Pletscher A (1977) Atropine induced changes of cerebral dopamine turnover. Experentia 27:1302-1303.

Brezenoff HE, Giuliano R (1982) Cardiovascular control by cholinergic mechanisms in the central nervous system. Ann Rev Pharmacol Toxicol 22: 341-381.

Brimblecombe RW (1977) Drugs acting on central cholinergic mechanisms and affecting respiration. Pharmacol Ther Part B 3:65-74.Corbier A, Robineau P (1989) Evidence for a direct noncholinergic effect of an organophosphorus compound on guineapig papillary muscles: are ventricular arrhythmias related to sodium-potassium ATPase inhibition ?. Arch Int Pharmacodyn Ther 300:218-230.

Domino EF, Wilson AE (1972) Psychotropic drug influence on brain acetylcholine utilization. Psychopharmacoiogia 25:291-298.

Dube SN, Kumar P, Kumar D, DasGupta S (1993) Route-specific cardio-respiratory and neuromuscular changes following organophosphate poisoning in rats. Arch Int Pharmacodyn Ther 321:112-122.

Franz DR, Hilaski R (1990) Sequence of cardiorespiratory effects of soman altered by routes of administration. Toxicol Lett 51:221-225.

Lang WJ, Rush ML (1973) Cardiovascular responses to injections of cholinomimetic drugs into the central vesicles of unanaesthetized dogs. Br J. Pharmacol 47:195-205.

Matin MA, Khan SN, Husain K, Sattar S (1989) Effect of adrenalectomy in diazinon-induced changes in carbohydrate metabolism. Arch Toxicol 63:376-380.

Preston E, Heath C (1972) Depression of vasomotor system in rabbits poisoned with an organophosphate anticholinesterase. Arch Int Pharmacodyn Ther 200:245-254.

Robineau P, Guittin P (1987) Effects of an organophosphorus compound on cardiac rhythm and haemodynamics in anaesthetized and conscious beagle dogs. Toxicol Lett 37:95-102.

Takahashi H, Kojima T, lkeda T, Tsuda S, Shirasu Y (1991) Differences in the mode of lethality produced through intravenous and oral administration of organophosphorus insectisides in rats. Fundam Appl Toxicol 16:459-468.

Willette RN, Punnen O, Kreiger AJ, Sapru HN (1984) Cardiovascular control by cholinergic mechanisms in thr rostral ventrolateral medulla. J Pharmacol Exp Ther 231:457-463.

Worek F, Kirchner T, Szinicz L (1995a) Effect of atropine and bispyridinium oximes on respiratory and circulatory function in guineapigs poisoned by sarin. Toxicol 95:123-133.

Worek F, Kleine A, Falke K, Szinicz L (1995b) Arrhythmias in organo-phosphate poisoning: effect of atropine and bispyridinium oximes. Arch Int Pharmacodyn Ther 329:418-435.

Worek F, Szinicz L (1995c) Cardiorespiratory function in nerve agent poisoned and oxime+atropine treated guineapigs: effect of pyridostigmine pretreatment. Arch Toxicol 69:322-329.

Yamamura HI, Snyder SH (1973) High affinity transport of choline into synaptosomes of rat brain. J Neurochem 21:1355-1374.