J Occup Health 2001; 43: 346–350

Journal of Occupational Health

Protective Effect of Clonidine against Toxicity of Organophosphorus Pesticides

Zhijun ZHOU, Yunping HU, Yunguang SUN and Jia CHEN

Department of Occupational Health and Toxicology, School of Public Health, Fudan University

Abstract: Protective Effect of Clonidine against Toxicity of Organophosphorus Pesticides: Zhijun ZHOU, *et al.* Department of Occupational Health and Toxicology, School of Public Health, Fudan University—Aim: The purpose of this study was to check whether clonidine, a centrally active alpha-2 adrenergic agonist, could serve as an antagonist against the acute toxicity of generally used organophosphorus pesticides (OP) in laboratory animals. Methods: The tests of three factors, the dose level of pesticide, and the administration schedules for atropine and clonidine, were arranged for cross-checking according to three level orthogonal layouts. The latency of onset of apparent body tremor, loss of righting reflex and survival time was timed. Results: The survival time was longest in the treatments with 5 mg/kg atropine, 20 min pre- OP injection or with 1 mg/kg clonidine, 10 min pre-OP injection. The latency of onset of apparent body tremor and the loss of righting reflex were prolonged when clonidine or atropine was given before OP administration. Conclusion: These results indicated that the use of clonidine in the clinical treatment of intoxication by means of organophosphates is reasonable and acceptable. It is a recommendation of a new use of an old drug. Used cautiously, beneficial effects of clonidine could be expected, either separately or combined with atropine, in the treatment of organophosphate intoxication.
(J Occup Health 2001; 43: 346–350)

Key words: Clonidine, Organophosphate, Experimental therapy

Organophosphorus pesticides are a diverse group of highly toxic chemicals to which workers may be exposed during manufacture and formulation as well as during or after application for their intended use. Intoxication due to these chemicals occurs very frequently in rural areas, since they are used extensively in agriculture and in an unsafe manner. This becomes a serious problem threatening the farmer's life and health, especially in developing countries where medical care in rural areas is insufficient[1].

All organophosphorus pesticides exert neurotoxicity via a common mechanism of action - binding to and phosphorylation of the enzyme acetylcholinesterase. This causes acetylcholinesterase inhibition and a buildup of neurotransmitter acetylcholine in the central and peripheral nervous system synapses resulting in overstimulation at muscarinic and nicotinic cholinergic synapses. Overstimulation at muscarinic synapses results in hypersalivation, excess lacrimation, miosis, intestinal cramps, vomiting, diarrhea, urinary and fecal incontinence, bronchorrhea and bronchoconstriction. Overstimulation at nicotinic synapses results in muscle cramps, fasciculation, weakness, paralysis and pallor. Central nervous system effects include anxiety, restlessness, dizziness, confusion, ataxia, convulsion and respiratory and circulatory depression[2].

The common remedies, combinations of atropine and oxime reactivator, such as 2-pyridine aldoxime methiodide (2-PAM), are effective in most cases, but fatalities in some severe cases with such therapies are still occurring. And the use of atropine must be meticulously done to prevent overdosing. Therefore, new drugs supplementary to or partly substituting for atropine are urgently needed to save lives.

Clonidine is a centrally active alpha-2 adrenergic agonist which is traditionally used as an anti-hypertensive drug in the clinic. Recently the preventive effects of clonidine against the toxicity of soman, an organophosphorus warfare toxicant, were reported[3–7]. These reports indicated that it is probable that clonidine could become an effective remedy for organo phosphorus insecticide poisoning.

The purpose of this study was to check whether clonidine could serve as an antagonist against the acute toxicity of generally used organophosphorus pesticides

Received April 2, 2001; Accepted Aug 27, 2001
Correspondence to: Z. Zhou, Department of Occupational Health and Toxicology, School of Public Health, Fudan University, 130 Dong An Road, Shanghai 200032, China

Table 1. Test program for orthogonal experiment design

Factor Level	Insecticide (mg/kg)	Atropine (5 mg/kg)	Clonidine (1 mg/kg)
1	Lethal dose 1 (lower)	No	No
2	Lethal dose 2 (middle)	20 min pre-OP injection	10 min pre-OP injection
3	Lethal dose 3 (higher)	10 min post-OPinjection	5 min post-OP injection

Table 2. The effect of atropine and clonidine on trichlorfon induced toxicity in mice

Factor	Level[1]	Loss of righting reflex (sec)	Latency of onset of body tremor (sec)	Survival time (min)
Trichlorfon	1 (800)[2]	73.5	50.1	186.7
	2 (1000)	71.9	52.5	90.1
	3 (1250)	85.5	52.0	19.3**
Atropine	1	71.4	47.3	33.0
	2	94.0	61.3	232.8
	3	65.6**	46.1**	30.2**
Clonidine	1	65.2	44.7	30.7
	2	93.6	63.7	188.7
	3	72.2**	46.3**	78.5**

[1] the indication of Levels 1, 2 and 3, referring to Table 1. [2] figures in brackets are the dosage levels of organophosphate (mg/kg). *p<0.05, **p<0.01, comparison of three levels of each factor.

in laboratory animals.

Materials and Methods

Organophosphorus pesticides and antidotes

Tricholofon (91.2%, technical grade), Dimethoate (85.6%, technical grade), Malathion (50% commercial product) and Dichlorovos (80% commercial product) were supplied by Shanghai Pesticide Factory. Methamidophos, 90% technical grade, was provided by Zhejiang Linhu Chemical Factory. Parathion, 78% technical grade, was provided by Jiangsu Qidong Pesticide Factory. They were diluted with distilled water except for dimethoate with isopropanol.

Atropine sulfate (0.5 mg/0.5 ml/ampoule) and clonidine hydrochloride (0.15 mg/2 ml/ampoule), preparation for injection was purchased from the pharmacy of Shanghai Ruijin Hospital.

Animal

Male Kunming mice, body weight 20–25 g, were supplied by the Department of Animal Science of Fudan University. They were kept for one week in this laboratory prior to the experiment.

Design of experiment

The orthogonal experiment design was employed in this study[8]. The tests of three factors — the dosage level of pesticides, the administration schedules for atropine and the administration schedules for clonidine, which affect the outcomes of treated animals, were crosscheck arranged according to three level orthogonal layouts. Five mice were used for each treatment. The exact design is shown in Table 1.

Treatment and observation

Mice were treated with chemicals according to the above mentioned schedules. All of the insecticides and drugs were intraperitoneally injected. After injection of tested organophosphorus insecticides, the mice were immediately returned to the observation cages and observed for the occurrence of overt signs and symptoms. The latency of onset of apparent body tremor, loss of righting reflex and survival time was timed with a stopwatch.

Analysis of data

Variation analysis for orthogonal experimental design was adopted[8].

Results

The results of tests for the three kinds of factors, organophosphorus insecticide, atropine and clonidine are shown in Table 2 to Table 6. The survival time for all tested organophosphorus insecticides was decreased as

Table 3. The effect of atropine and clonidine on dimethoate-induced toxicity in mice

Factor	Level[1]	Loss of righting reflex (sec)	Latency of onset of body tremor (sec)	Survival time (min)
Dimethoate	1 (400)[2]	97.1	–	608.8
	2 (800)	49.0	–	323.9
	3 (1200)	45.5**	–	58.7**
Atropine	1	57.4	–	115.3
	2	69.6	–	525.7
	3	64.6**	–	350.4**
Clonidine	1	51.5	–	153.1
	2	84.5	–	403.5
	3	55.6**	–	437.4**

[1] the indication of Levels 1, 2 and 3, referring to Table 1. [2] figures in bracket are the dosage levels of organophosphate (mg/kg). *p<0.05, **p<0.01, comparison of three levels of each factor.

Table 4. The effect of atropine and clonidine on malathion induced toxicity in mice

Factor	Level[1]	Loss of righting reflex (sec)	Latency of onset of body tremor (sec)	Survival time (min)
Malathion	1 (500)[2]	62.1	108.1	19.2
	2 (1000)	54.0	95.9	17.1
	3 (2000)	66.7*	119.5*	14.6**
Atropine	1	61.5	102.6	17.1
	2	62.0	120.7	19.4
	3	59.0**	100.1**	14.3**
Clonidine	1	51.6	99.1	17.1
	2	77.2	130.9	20.7
	3	54.0**	93.5**	13.1**

[1] the indication of Levels 1, 2 and 3, referring to Table 1. [2] figures in brackets are the dosage levels of organophosphate (mg/kg). *p<0.05, **p<0.01, comparison of three levels of each factor.

Table 5. The effect of atropine and clonidine on methamidophos, induced toxicity in mice

Factor	Level[1]	Loss of righting reflex (sec)	Latency of onset of body tremor (sec)	Survival time (min)
Methamidophos	1 (800)[2]	571.8	447.1	288.5
	2 (1000)	383.8	325.7	12.3
	3 (1250)	268.5**	215.6**	10.7**
Atropine	1	373.9	327.1	11.8
	2	482.6	357.3	286.1
	3	367.7**	304.1**	12.9**
Clonidine	1	304.1	257.5	10.3
	2	595.1	459.3	290.7
	3	327.1**	271.6**	10.6**

[1] the indication of Level 1, 2 and 3, referring to Table 1. [2] figures in brackets are the dosage levels of organophosphate (mg/kg). *p<0.05, **p<0.01, comparison of three levels of each factor.

Table 6. The effect of atropine and clonidine on dichlorovos induced toxicity in mice

Factor	Level[1]	Loss of righting reflex (sec)	Latency of onset of body tremor (sec)	Survival time (min)
Dichlorovos	1 (200)[2]	39.7	31.7	483.7
	2 (400)	34.6	27.2	11.8
	3 (600)	34.2*	27.0*	9.2**
Atropine	1	33.1	26.9	8.5
	2	39.5	31.1	487.3
	3	36.0**	27.9**	6.9**
Clonidine	1	35.1	27.9	7.9
	2	45.1	34.8	487.3
	3	28.4**	23.2**	7.7**

[1] the indication of Levels 1, 2 and 3, referring to Table 1. [2] figures in brackets are the dosage levels of organophosphate (mg/kg). *$p<0.05$, **$p<0.01$, comparison of three levels of each factor.

the dosage of insecticide increased. The survival time was longest in the treatments with 5 mg/kg atropine, 20 min pre- OP injection or with 1 mg/kg clonidine, 10 min pre-OP injection. The whole body tremor for some organophosphates, such as DDVP, was very quick and prominent; but for the others, such as dimethoate, was not significant. The latency of onset of body tremor and loss of righting reflect for trichlofon did not shorten as the dosage increased.

Discussion

The purpose of this study was to observe and compare the preventive effects of atropine and clonidine given in different time courses in the poisoning of mice with organophosphates. The orthogonal experimental design was used to balance the confounding factors and minimize the number of animals tested. The figures showed in the tables were not the actual numerical data, but the integrated parameters, derived from these three tested factors, were only used for comparison[8].

Whole body tremor for some insecticides, such as DDVP, occurred very quickly and prominently, but for others, such as Dimethoate, it was not significant. This indicated the difference between intoxication symptoms and signs among the time-effect courses of different organophosphates. The survival time of mice tested with all five insecticides was decreased as the dosage level increased, which verified the truth of this study, but the onset latency of body tremor and loss of righting reflect with trichlofon was not reduced as the toxicant dosage was increased. This may be due to the existence of a threshold[2].

It is well known that acute effects of organophosphates are due to the inhibition of hydrolysis of acetylcholine by acetylcholinesterase. This results in cholinergic effects due to an excess of acetylcholine accumulated at the cholinergic synapses, thus leading to a variety of muscarinic and nicotinic manifestations[2]. Atropine is a muscarinic cholinergic blocking agent, which competitively inhibits acetylcholine at the parasympathetic, postganglionic nerve endings. Therefore, it is a specific antidote for organophosphate poisoning and has obvious effectiveness in the treatment of intoxication in the clinic. Nevertheless, while the need for muscarinic blockade in this situation is clear-cut, there is controversy as to the most appropriate dosage regimen for atropine, though the position of atropine in the treatment of organophosphate poisoning is essentially unchallenged[9].

Buccafusco and Aronstam were the first to report that pretreatment with clonidine protects against several of the centrally- mediated toxic effects of soman, increasing survival rates[3]. Since then several other reports have been published which show the effectiveness of clonidine[4–7].

The results of our study, shown in the Tables 2 to 6, indicate that both atropine and clonidine could increase the survival time of intoxicated mice. Pretreatment with atropine or clonidine could postpone the occurrence of whole body tremor and the loss of righting reflex in poisoned mice. The results showed that the sooner the drugs were given, the greater the therapeutic effectiveness noticed.

Clonidine has multiple pharmacological actions, most of which are triggered by stimulation with alpha-2 adreno-receptor[10]. Clonidine can inhibit the release of acetylcholine from postganglionic parasympathetic and preganglionic sympathetic nerve terminals. The inhibition of acetylcholine synthesis and release by clonidine in certain cerebral regions has also been demonstrated, but it was found that clonidine has a protective effect against the centrally acting cholinesterase inhibitor, physosstigmine, but not the peripherally acting

cholinesterase inhibitor, neostigmine. Clonidine is presumably acting as a central presynaptic cholinergic antagonist, but atropine is only a peripheral postsynaptic muscarinic antagonist. Because there is a synergistic effect when clonidine is combined with atropine, it is reasonable to expect that they could play roles supplementary to each other and enhance the effectiveness of therapy.

In conclusion, the use of clonidine in the clinical treatment of intoxication with organophosphates sounds reasonable and acceptable, since this drug has been used to treat hypertension for many years without substantially adverse effects. It is the recommendation of a new use for an old drug. Used cautiously, beneficial effects of clonidine could be expected, either separately or combined with atropine, in the treatment of organophosphate intoxication.

Acknowledgment: This work was supported by a grant from the State Ministry of Education of China (special funds for university, number 200040).

References

1) Jeyaratnam J, Maroni M. Organophosphorus compounds. Toxicology 1994; 91: 15–27.
2) Koelle GB. Pharmacology and Toxicology of Organophosphates. In: Ballantyne B & Marre TC eds. Clinical and Experimental Toxicology of Organophosphates and Carbamates. Oxford: Butterworth/ Heiman, 1992: 40–46.
3) Buccafusco JJ, Aronstam RS. Clonidine protection from the toxicity of soman, an organophosphate acetylcholonesterase inhibitor, in the mouse. J Pharmacol Exp Ther 1986; 239: 43–47.
4) Liu WF. A symptomatological assessment of organophosphate induced lethality in mice: comparison of atropine and clonidine protection. Toxicology Letter 1991; 56: 19–32.
5) Shih T, McDonough JH, Koplovitz I. Anticonvulsants for soman-induced seizure activity. J Biomed Sci 1999; 6: 86–96.
6) Magri V, Buccafusco JJ, A ronstam RS. Protection by clonidine from cardiovascular changes and lethality following soman administration in the rat: role of brain acetylcholine. Toxicol Appl Pharmacol 1988; 95: 464–473.
7) Delaunois A, Gustin P, Vargas M, Ansay M. Protective effect of various antagonists of inflammatory mediators against paraoxon-induced pulmonary edema in the rabbit. Toxicol Appl Pharmacol 1995; 132: 343–345.
8) Zhan SK. Variance analysis of orthogonal experiment design. In: Jin PH, Zhan SK, Zhang ZH, eds. Medical Statistics. Shanghai: Shanghai Medical University Press, 1993: 85–99.
9) Heath AJW, Meredith T. Atropine in the management of anticholinesterase poisoning. In: Ballantyne B & Marre TC eds. Clinical and Experimental Toxicology of Organophosphates and Carbamates. Oxford: Butterworth/ Heiman, 1992: 543–554.
10) Lin ZB, Jin YY, eds. Basis of Medical Pharmacology (the National University Textbook, 4th Edition). Beijing: the Century Book Publisher Company, 1998.