

## INFLUENCE OF CLONIDINE, METHYLDOPA AND PROPRANOLOL ON ACUTE TOXICITY OF FENITROTHION IN MICE

N. VALECHA, S. PRABHU AND V. L. MEHTA\*

Department of Pharmacology,  
Maulana Azad Medical College,  
New Delhi - 110 002

(Received on August 17, 1989)

**Abstract :** The effect of pretreatment with clonidine, methyldopa and propranolol, and of atropine was studied in mice on acute toxicity of fenitrothion, the active ingredient of TIK-20. Atropine significantly decreased and propranolol somewhat decreased the fenitrothion induced death in mice. Clonidine and methyldopa somewhat increased the percentage mortality due to fenitrothion.

**Key words:** fenitrothion                      anticholinesterase                      lethality

### INTRODUCTION

Fenitrothion [0,0-dimethyl-0-(3-methyl-4-nitrophenyl), phosphorothioate], the active ingredient of TIK-20 is an irreversible cholinesterase inhibitor and is used as a contact insecticide, and domestic pesticide. It would produce symptoms of typical cholinergic poisoning, involving central as well as peripheral cholinergic system in animals (1). Pretreatment with atropine is reported to increase the LD<sub>50</sub> of fenitrothion significantly (2).

Presently, the only class of drugs employed as antidotes for cholinesterase poisoning are targeted on postsynaptic components of cholinergic transmission. These include muscarinic receptor blocking agents to inhibit actions of accumulated acetylcholine (Ach) in peripheral and central tissues and cholinesterase reactivators which are employed for organophosphate type irreversible inhibitors (3). Very few drugs are available which can be used to block the release of Ach from the nerve ending, an action which would theoretically be useful (3) in such poisonings. It has been reported by Buccafusco (3) that clonidine could inhibit the turnover rate of brain Ach which is mediated through  $\alpha$ -adrenergic receptors. They also proposed that as with peripheral visceral efferent cholinergic neurons, the  $\alpha$ -adrenergic receptors may be located on central cholinergic nerve endings. Clonidine has been shown to protect against physostigmine (a central and peripheral cholinesterase inhibitor) induced

tremors and respiratory depression which was reversed by yohimbine. This proves that protective effect of clonidine was mediated through central  $\alpha_2$ -receptors. Respiratory paralysis induced by neostigmine, which predominantly inhibits peripheral cholinesterase was not affected by clonidine.

Aronstam et al (4) have reported that pretreatment with clonidine protects against toxic manifestations of soman but has little effect on echothiophate-induced toxicity in mice. Toxicity of soman and echothiophate primarily reflects central and peripheral actions respectively and clonidine has a much greater protective effect against the centrally active agents.

Methyldopa, like clonidine, has been shown to inhibit brain Ach biosynthesis in spontaneously hypertensive rats (5). It reduces Ach turnover in selective brain regions. Whether methyldopa also has a protective effect like clonidine, against central toxic effects of anticholinesterases has not been elucidated so far.

Propranolol is known to reduce the noradrenaline release during nerve stimulation in several tissues by blocking presynaptic  $\beta$ -adrenoceptors that mediate positive feed back mechanism for noradrenaline release (6). The effect of propranolol on Ach biosynthesis in the brain has so far not been reported. Propranolol could serve to elucidate whether the protective effects of clonidine against anti-cholinesterase poisoning lies in

\*Corresponding Author



its action on central adrenergic receptors or on cholinergic transmission, since it lacks the latter action.

The present study was hence planned to elucidate the effect of clonidine, methyl dopa and propranolol on fenitrothion induced toxicity.

### METHODS

Only male mice weighing 25-35 gms, fed on standard diet (Hindustan Lever Feed) were used in all the experiments since fenitrothion has different LD<sub>50</sub> values (7) in males (1045 mg/kg, po) and females (1220 mg/kg, po). Animals were allowed access to food and water ad libitum.

Clonidine, methyl dopa, propranolol and atropine were dissolved in distilled water while fenitrothion was dissolved in propylene glycol. It was ascertained *a priori* that propylene glycol in the amount used as a solvent did not have any toxic effect *per se*.

Fenitrothion (ip) was administered in increasing doses in different groups (n=10) and the mice were then placed in individual clear plastic cages for observation. A dose near to LD<sub>50</sub> (300 mg/kg) was used to elucidate the effect of drug pretreatments (see results).

Clonidine (0.3 and 1.0 mg/kg, ip) was administered 20 min prior, while methyl dopa (50 and 200 mg/kg, ip) and propranolol (5 and 10 mg/kg, ip) were administered 1 hr prior to fenitrothion. Atropine (5 mg/kg, ip) was

used as a standard (protective) reference drug and was given just before fenitrothion.

The parameters which were noted in all the experiments were occurrence of straub tail (ST), muscle fasciculation (MF) and excessive salivation. In addition, time to loss of righting reflex (LRR) (4) and mortality at one as well as at 24 hr was also noted: the latter was to cover possible residual effects (1).

Statistical analysis of mortality data was done by Chi<sup>2</sup> test and rest of the data was analysed by 't' test.

### RESULTS

*Effect of fenitrothion in mice:* The lethality dose curve of fenitrothion covered very wide dose range. Percent mortality at 24 hrs with different doses of fenitrothion viz., 150, 300, 500 and 750 mg/kg was 16.66, 53.96, 70 and 100% respectively. Effect of drug pretreatment was studied using a single dose of fenitrothion, viz., 300 mg/kg which is near to LD<sub>50</sub>. Since the lethality of this dose varied in different batches of animals (40-87.5% at 24 hr), a control group was run with every drug pretreatment. This also served to eliminate the bias due to factors like temperature and humidity. Neither LRR nor the incidence of other parameters, like ST or MF showed any correlation with the dose of fenitrothion used.

*Effect of atropine on fenitrothion toxicity:* Atropine decreased the lethality of fenitrothion (Table I). There

TABLE I : Influence of atropine, clonidine, methyl dopa and propranolol on fenitrothion toxicity in mice.

Drug	Dose mg/kg ip	n	Lethality		No of animals showing LRR	LRR (min) ±SEM	MF %	ST %
			1hr	24hr				
Control	-	20	25	80	19	10.05±0.55	15	30
Atropine	5.0	18	5.55	27.77*	17	13.70±0.56	5.55	22.22
Control	-	10	20	50	8	12.37±2.16	10	20
Clonidine	0.3	10	0	70	8	16.62±2.11	10	10
Control	-	10	10	40	6	12.33±2.23	20	20
Clonidine	1.0	10	10	80	9	17.22±2.92	40	10
Control	-	10	40	80	10	15.8±1.87	20	0
M-Dopa	50	10	70	90	9	17.4±5.05	10	0
Control	-	10	0	50	6	16.76±2.53	40	60
M-Dopa	200	10	30	90	8	21.5±3.83	10	30
Control	-	8	12.5	87.5	8	16.75±2.53	50	12.5
Propranolol	5	8	37.5	62.5	7	14.14±2.6	12.5	12.5
Control	-	10	30	80	9	16.25±2.73	20	10
Propranolol	10	10	30	50	7	18.14±2.09	30	10

Atropine was administered just prior, clonidine 20 min prior, and methyl dopa and propranolol 1 hr prior to 300 mg/kg fenitrothion (ip) LRR, time to loss of righting reflex; MF, occurrence of muscle fasciculations; ST, occurrence of straub tail.

\*P < 0.01 ('t' test)



was a slight, insignificant decrease in the incidence of other parameters of toxicity (MF, ST).

*Effect of clonidine, methyldopa and propranolol on fenitrothion toxicity* : Clonidine and methyldopa somewhat increased while propranolol somewhat decreased the 24 hr mortality in mice due to fenitrothion in both the doses employed. However, the changes were not statistically significant ( $P > 0.05$ ). Other parameters did not significantly change in drug-treated groups.

### DISCUSSION

Aronstam et al (4) used the onset latency to LRR as a measure of survival time, since they found it to be more sharply defined than other indications of death. Loss of heart beat always followed LRR within 90 sec in their experiments. We observed no such correlation. Further there was no correlation between dose of fenitrothion injected and the toxic effects like LRR, MF and ST. This is in contrast to observations on soman and echothiophate (4).

Our results with atropine are comparable with those of other workers (2).

Clonidine reduces lethality, increases LRR, reduces the incidence of ST and excessive salivation in mice injected with  $LD_{50}$  of soman but not echothiophate (4). We now find that clonidine has no major effect against fenitrothion.

The effect of methyldopa on toxic effects of fenitrothion or any other anticholinesterase has not been reported so far. Methyldopa in the doses employed was found to delay to some extent the onset to LRR,

decrease the incidence of MF and in the dose of 200 mg/kg, decrease the incidence of ST, though these effects were statistically not significant ( $P > 0.05$ ).

Unlike clonidine and methyldopa, propranolol had no tendency to increase the lethality of fenitrothion though occurrence or worsening of the broncho constriction produced by an organophosphorus cholinesterase inhibitor could be expected after propranolol. Lack of effect of propranolol on fenitrothion lethality suggests that the latter is unrelated to central  $\beta$ -adrenergic mechanisms.

Lack of protective effect of all centrally acting drugs against fenitrothion toxicity observed in the present study could be due to inability of drugs to reverse the peripheral effects of fenitrothion as has been reported earlier in the case of echothiophate (4) and neostigmine (3). It is also possible that the drugs are not potent enough in reducing Ach turnover, though clonidine (6) and methyldopa (5) have such an action. It is difficult to explain why clonidine and methyldopa tend to increase and propranolol tends to decrease the lethality due to fenitrothion.

Although the present study clearly indicates that these drugs may not alter the acute toxicity of fenitrothion, it is possible that they may have at least some prophylactic value in chronic organophosphorus insecticide toxicity, which needs to be explored.

### ACKNOWLEDGEMENTS

The authors gratefully acknowledge the free gift of fenitrothion by Rallis India Ltd., Clonidine by S.G. Pharmaceuticals and methyldopa by IDPL.

### REFERENCES

- Wayland JG, Organophosphorus insecticides. In : Pesticides studied in man. *New York: Williams & Wilkins* 1982; 365-7.
- Saxena RK, Mishra NP, Gupta SS. Experimental study of TIK-20 poisoning and its treatment. *Ind J Physiol Pharmac* 1974; 18:212.
- Buccafusco JJ. Mechanism of Clonidine induced protection against acetyl cholinesterase inhibitor toxicity. *J Pharmacol Exp Ther* 1982; 22: 595-9.
- Aronstam RS, Smith MD, Buccafusco JJ. Clonidine protection from Soman and Echothiophate toxicity in mice. *Life Sci* 1986, 39: 2097-102.
- Buccafusco JJ. Inhibition of brain acetylcholine biosynthesis by methyldopa in spontaneously hypertensive rats. *Brain Res Bull* 1984; 13: 257-62.
- Clark WG, Brater DC, Johnson AR. Adrenergic blocking agents In : Goth's Medical Pharmacology. *Galgotia Publications* 1989; 163-71.
- Haley TJ, Farmer JH, Harmon JR, Dooley KL. Estimation of  $LD_1$  and extrapolation of  $LD_{01}$  for five organothiophosphates. *Pesticides J Eur Toxicol* 1975; 34: 103-9.