Effects of Atropine Sulphate Treatment on Hepatic Mixed Function Oxidase System during Baygon Intoxication

Surinder J. Makhija and Sitaram S. Pawar
Biochemistry Division, Chemistry Department
Marathwada University
Aurangabad, Maharashtra, India

Changes in the toxicity of insecticides like Baygon, Zectran can occur through treat ment with atropine sulphate or tetraethylammoniumbromide(VERSCHOYLE and BARNES 1969, KOHGO and IKEDA 1967). ZELETIN and POPESCU(1967) have observed atropine to be an antitoxic agent against organophosphorus compounds. ANDREWS and MISKUS(1968) have found antidotal effect of tetraethylammoniumchloride to be more effective than atropine sulphate in mice, whereas, the reverse effect was observed by KIMMERLE(1971) in rats. Recently, it has been reported that administration of atropine sulphate prolonged thiopental sleeping time in dogs(KLIDE et al. 1974). It has also been reported that atropine sulphate induced paraoxon sleeping time in mice(KOEPKE et al. 1974).

Literature rewiew reveals a paucity of data regarding protective action of atropine sulphate on hepatic drug metabolism during Baygon intoxication and hence the present studies were undertaken to investigate such observations.

MATERIALS AND METHODS

Young male and female C.F. strain albino rats initially weighing 55-65 gms were obtained from M/s Ghosh and Co.,Calcutta. The animals were housed in individual cages in an air conditioned room and supplied with standard rat pellets (obtained from Hindustan Lever Ltd., Bombay) and water ad libitum for 10-15 days prior to the initiation of the experiments. The animals were then divided into the following five groups each containing 5-6 animals.
1) Control group, 2) Baygon treated group, 3) Atropine sulphate treated group, 4) Baygon followed by atropine sulphate treated group and 5) Atropine sulphate followed by Baygon treated group.

INJECTION SCHEDULE

Baygon (10 mg/kg in corn oil, ip) was

Bulletin of Environmental Contamination & Toxicology,
Vol. 16, No. 1 © 1976 by Springer-Verlag New York Inc.

administered to rats daily in the morning for two successive days. Atropine sulphate (5 mg/kg) was injected intraperitoneally for two days. The The animals from IV group were injected first with Baygon for two days and then with atropine sulphate for another two days. The animals from V group received atropine sulphate for two days followed by Baygon for two days. The control animals received equivalent amount of corn oil.

TISSUE PREPARATION AND DRUG METABOLISM

The animals were sacrificed 24 hours after the last injection in each group by decapitation. The livers were removed by perfusion with 0.9% ice cold saline, weighed, a part of them were minced and homogenized(1:4 w/v) in ice cold 0.25 M sucrose with a teflon pestle glass homogenizor. The homogenates were centrifuged at 9000xg for 20 minutes in a Remi K-24 centrfuge. The 9000xg supernatant fraction was further used for drug assays.

A second portion of the remaining livers were homogenized (1:4 w/v) in ice cold 50 mM Tris-HCl buffer pH 7.4 containing 1.15% KCl. The microsomes were isolated as reported by Baker et al.(1973). The microsomal pellets were resuspended in 0.25 M sucrose. The 9000xg supernatant fraction and microsomal proteins were estimated according to the biuret method(GORNALL et al. 1949) using crystalline bovine serum albumin as the standard.

The 9000xg drug metabolizing enzymes were measured as reported earlier(MAKHIJA and PAWAR 1974) using aminopyrine, ethylmorphine, methylaniline, aniline and acetanilide as substrates. The levels of microsomal cytochrome b5, NADH cytochrome c reductase, total heme and pyridine binding spectra were determined as reported earlier (PAWAR and MAKHIJA 1975).

RESULTS

During Baygon intoxication, the relative liver weights decreased by only 1.5% in male rats and increased by 8.5% in female rats. When male rats were treated with atropine sulphate there was no change in relative liver weights, however, 6.8% increase was noticed in female rats. Atropine sulphate treatment before and a after Baygon intoxication resulted in an increase in relative liver weights irrespective of sex variation (TABLE 1).

During Baygon intoxication the decrease in 9000xg supernatant protein content was 10.6 and 13.9 per

TABLE 1

Effect of atropine sulphate on relative liver weights and protein content during Baygon intoxication in young growing rats.

Group	Sex	Relative liver weights (gms)	9000xg supernatant protein (mg/gm liver)	microsomal protein
Control	Male	3.96	179.4	43.0
	Female	4.12	204.9	45.2
Baygon	Male	3.90^{NS}	160.3^{a}	54.6^{b}
	Female	4.47^{a}	176.3^{a}	28.6^{c}
Atropine sulphate	Male	4.00^{NS}	172.2^{a}	45.2^{a}
	Female	4.40^{a}	211.4^{b}	41.0^{a}
Baygon+ atropine sulphate	Male	4.13^{a}	173.1^{NS}	49.4^{a}
	Female	5.60^{c}	192.6^{c}	44.2^{NS}
Atropine sulphate+ Baygon	Male	4.04^{NS}	181.0^{NS}	46.2^{a}
	Female	5.23^{b}	229.1^{a}	43.2^{a}

Values represent mean of 3 determinations from 5 rats in each group.
a = $P<0.05$; b = $P<0.01$; c = $P<0.001$; NS= not significant.

cent in male and female rats respectively. On the contrary, the hepatic microsomal protein content increased by 27.0% in male and decreased by 36.7% in female rats. A 4.1% decrease in 9000xg supernatant fraction protein content was noted in male rats treated with atropine sulphate and 17.0% increase in female rats. However, the hepatic microsomal protein content increased by 5.1% in male rats and decreased by 9.2% in female rats.Atropine sulphate treatment after Baygon intoxication resulted in a decrease in 9000xg supernatant protein content, whereas, treatment of rats with atropine sulphate before Baygon intoxication revealed an increase. However, the magnitude of increase or decrease was significantly high in female as compared to the male rats. The result of atropine sulphate before and after Baygon intoxication was 7.44% and 14.8% increase in the respective microsomal protein of male rats,however, a slight decrease in the microsomal protein content was noted in the case of female rats.

TABLE 2. Effect of atropine sulphate treatment during Baygon intoxication on drug metabolizing enzyme activities in young growing rats.

Group	Sex	N-Demethylation *			Hydroxylation**	
		Aminopyrine	Ethylmorphine	Methylanilne	Aniline**	Acetanilide***
Control	Male	15.0±0.3	12.9±0.3	9.1±0.2	4.0±0.1	1.23±0.01
	Female	7.5±0.2	6.2±0.1	4.4±0.2	3.0±0.1	0.76±0.01
Baygon	Male	12.5±0.2^{b}	11.6±0.3^{a}	8.1±0.2^{a}	3.2±0.2^{b}	1.0±0.01^{b}
	Female	6.2±0.2^{b}	5.6±0.2^{a}	3.1±0.1^{b}	2.6±0.1^{a}	0.64±0.02^{a}
Atropine sulphate	Male	15.8±0.1^{a}	12.2±0.2^{a}	8.5±0.1^{a}	3.8±0.1^{a}	1.16±0.01^{a}
	Female	6.0±0.1^{b}	5.0±0.2^{b}	3.5±0.1^{b}	2.5±0.2^{b}	0.68±0.02^{a}
Baygon + Atropine sulphate	Male	17.4±0.7^{b}	15.5±0.1^{b}	11.0±0.1^{b}	3.6±0.1^{a}	0.97±0.01^{b}
	Female	8.1±0.2^{a}	7.5±0.1^{b}	4.6±0.1^{a}	2.9±0.1^{a}	0.77±0.01^{NS}
Atrophine sulphate + Baygon	Male	19.3±0.4^{b}	15,9±0.2^{b}	11.5±0.2^{b}	3.4±0.2^{a}	1.14±0.01^{a}
	Female	6.6±0.2^{a}	4.2±0.3^{c}	3.8±0.1^{a}	2.9±0.1^{a}	0.73±0.01^{a}

Values are the means ± S.E.M. (5 rats in each group)
* = nM formaldehyde produced/min/mg protein.
** = nM p-aminophenol produced/min/mg protein.
*** = µM p-OH-acetanilide produced/min/mg protein.

NS = Not significant.
a = $P < 0.05$
b = $P < 0.01$
c = $P < 0.001$

A significant decrease in aminopyrine, ethylmorphine and methylaniline N-demethylase activities was observed during Baygon intoxication. The per cent decrease in the activities of aminopyrine and ethylmorphine was 16.0 and 10.0 respectively in animals of both the sexes. However, the per cent decrease in methylaniline N-demethylase activity was 8.7 in male and 28.5 in female rats. The aniline and acetanilide hydroxylase activities decreased by 20.0% and 12.2% in male rats and 13.3% and 16.8% in female rats during Baygon intoxication. Atropine sulphate treatment increased the aminopyrine N-demethylase activity by 5.3% in male and reduced by 20.0% in female rats. The per cent decrease in ethylmorphine and methylaniline N-demethylase activities was 5.4 and 6.6 in male and 20.0 and 19.4 in female rats.However, the decrease in aniline and acetanilide hydroxylase activities of male rats was only 5.0 and 5.6 per cent as compared to 16.7 and 10.5 per cent in female rats. Intoxication of Baygon before and after atropine sulphate treatment resulted in an increase in the activities of aminopyrine, ethylmorphine and methylaniline N-demethylase in male rats. However, administration of atropine sulphate after Baygon intoxication increased the N-demethylase activities in female rats, whereas, administration of atropine sulphate before Baygon intoxication resulted in a decrease. The aniline and acetanilide hydroxylase activities were decreased during Baygon intoxication before and after atropine sulphate treatment in either sex.

During Baygon intoxication the levels of cytochrome b_5 decreased by 17.5% in male rats and increased by 42.5% in female rats. In male rats 10.0% increase in cytochrome c reductase was noted, whereas, there was 15.0% decrease in female rats.During Baygon intoxication the total heme content increased by 10.0% and 67.5% in male and female rats respectively.

Treatment of rats with atropine sulphate resulted in an increase in cytochrome b_5 and cytochrome c reductase by 7.5% and 50.0% in male rats and 7.5% and 32.5% in female rats. The total heme content decreased by 2.5% in male and increased by 32.5% in female rats. The per cent change in electron transport components during atropine sulphate treatment before and after Baygon intoxication was as follows:

		ASO_4+Baygon	Baygon+ASO_4
cytochrome b_5	Male	- 10.0	- 10.0
	Female	+ 32.5	+ 32.5
cytochrome c reductase	Male	+ 10.0	+ 40.0
	Female	- 7.5	+ 50.0
total heme	Male	- 22.5	- 40.0
	Female	- 10.0	- 17.5

TABLE 3

Effect of atropine sulphate treatment on electron transport components during Baygon intoxication in young growing rats.

Group	Sex	cytochrome b_5*	cytochrome c reductase**	total heme*
Control	Male	0.240	60.0	0.77
	Female	0.150	64.0	0.46
Baygon	Male	0.200^{b}	66.0^{a}	0.84^{a}
	Female	0.215^{c}	54.0^{a}	0.77^{c}
Atropine sulphate	Male	0.255^{a}	90.0^{c}	0.75^{a}
	Female	0.160^{a}	84.0^{c}	0.61^{c}
Baygon+ atropine sulphate	Male	0.220^{a}	84.0^{c}	0.46^{c}
	Female	0.200^{c}	96.0^{c}	0.38^{c}
Atropine sulphate+ Baygon	Male	0.220^{a}	66.0^{a}	0.60^{b}
	Female	0.200^{c}	60.0^{a}	0.42^{a}

*= nM/mg protein
**= nM/min/mg protein
a = $P < 0.05$; b = $P < 0.01$; c = $P < 0.001$.

During Baygon intoxication the magnitude of microsomal pyridine binding spectra decreased by 9.1% and 32.2% in male and female rats respectively. However, the magnitude of pyridine binding spectra was not affected with atropine sulphate treatment in male rats, whereas, in female rats 20.0% increase was noted. In male rats the decrease in the magnitude of pyridine binding spectra during intoxication of Baygon before and after atropine sulphate was 22.2% in both cases, whereas, in female rats the decrease was 33.3% before atropine sulphate and 6.6% after atropine sulphate(Fig 1).

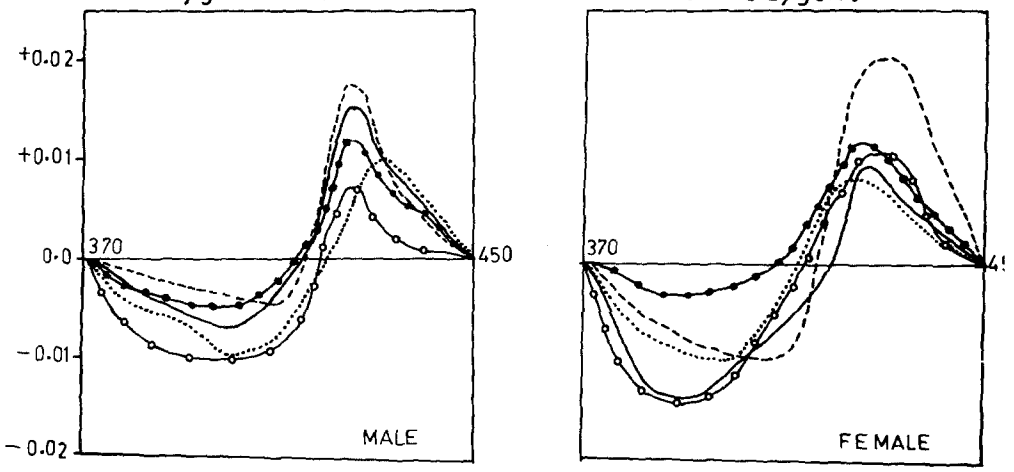
DISCUSSION

Although Baygon intoxication resulted in a decrease of relative liver weights and no change with atropine sulphate in male rats, net result of Baygon and

Fig: 1. Effect of atropine sulphate (AS04) treatment during Baygon intoxication on pyridine binding spectra in young growing rats.

——— Control ..... Baygon - - - - - - - Atropine sulphate

—•—•— Baygon+AS04 —o—o— AS04 + Baygon.



atropine sulphate was an increase which is due to the interaction of Baygon and atropine sulphate. Generally an increase in the microsomal protein/gm liver is associated with an increase in the drug metabolizing enzymes. However, in the present studies it was observed that although protein content decreased there was an increase in the drug enzymes. Similar observations have been reported by CRESS and STROTHER(1974).

The decrease in the activities of drug metabolizing enzymes during Baygon intoxication is due to hydroxy Baygon, a major metabolite of Baygon produced in the body. The observed higher levels of drug metabolzing enzymes during atropine sulphate and Baygon intoxication may be due to the change in the active sites of the enzymes and observed change in electron transport components. WELCH and COON(1964) proposed that the increase in resistance to organophosphate toxicity was related to an increased production of new binding sites in the liver. These binding sites could remove the insecticides from the circulation. FASTIER et al.(1957) found that atropine sulphate (10 mg/kg) alone significantly increased the loss of righting reflex time (LORRT) of chloral hydrate in mice. However, PROCTOR et al.(1964) observed that atropine sulphate counteracted the prolongation of LORRT in mice. The mechanism of protection offered by atropine sulphate is yet to be fully explored. A single dose of atropine was found to relieve the effects of carbamate insecticides both in rats and mice(VERSCHOYLE and BARNES 1969).

ACKNOWLEDGMENT

This research is partially supported by the Council of Scientific and Industrial Research, New Delhi. SJM is a CSIR, junior research fellow. SSP is grateful to Prof.R.W.Estabrook, Professor and Chairman, Department of Biochemistry, Health Science Center, University of Texas, Dallas, Texas, USA for his help and encouragement during the work. The authors also wish to thank Dr.J.M.Patel (presently at NIEHS, Research Triangle Park, North Carolina, USA) for his help and encouragement during the earlier stages of the work. We also wish to extend our thanks to Bayer(India) Limited for the gift of Baygon.

REFERENCES

ANDREWS,T.L. and MISKUS,R.P.: Nature.159,1367(1968).
BAKER,R.C., COONS,L.B. and HODGSON,E.: Chem-Biol. Interac.6,307(1973).
CRESS,C.R. and STROTHER,A.:Life Scien.14,861(1974).
FASTIER,F.N., SPEDEN,R.N. and WAAL,H.: Brit.J.Pharmacol.12,251(1957).
GORNALL,A.G., BARDAWILL,C.J. and DAVID,M.M.: J.Biol. Chem.177,751(1949).
KIMMERLE,G.: Arch.Toxicol.27,311(1971).
KLIDE,A.M., RIVAS,C. and PETERS,J.: J.Amer.Vet.Med. Assoc.164,1029(1974).
KOEPKE,U.C., COON,J.M. and TRIOLO,A.J.: Toxicol. Appl.Pharmacol.30,36(1974).
KOHGO,K. and IKEDA,Y.: Show.Yaka.Daigaku.Kujo. 5,9(1967).
MAKHIJA,S.J. and PAWAR,S.S.: Ind.J.Biochem.Biophys. 11,266(1974).
PAWAR,S.S. and MAKHIJA,S.J.: Bull.Environ.Contam. Toxicol.14,197(1975).
PROCTOR,D.C., RIDLON,S.A., FUDEMA,J.J. and PRABHU,U. G.: Toxicol.Appl.Pharmacol.6,1(1964).
VERSCHOYLE,R.D. and BARNES,J.M.: Bull.WHO.41,306(1969).
WELCH,R.M. and COON,J.M.: J.Pharmacol.Exper.Ther. 144,192(1964).
ZELETIN,P.I. and POPESCU,M.: Farmacia(Bucharest). 15,421(1967).