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Alterations in the Toxicity of Thiodemeton Due to the Pretreatment of Inducers, Substrates and Inhibitors of Mixed Function Oxidase System

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The toxicity of pesticides and other environmental chemicals may be altered by pretreatment with inducers of hepatic microsomal drug metabolizing enzyme system (BRODEIUR 1967, MENZER AND BEST 1968). Since the presence in harvested crops, of small quantities of the pesticides used in the agriculture is frequently unavoidable, information is needed on the extent to which enzyme induction or inhibition alters the toxicity of pesticides. Previous studies on mammalian toxicity or organophosphorus insecticides have indicated that this class of compounds interferes with many parameters involved in the metabolic balance of the organism (GAGE 1953, GAINES et al 1967, ROSENBERG and COON 1958, WELCH et al 1959, 1967, GAVAGNA et al 1969, MURPHY and CHEEVER 1972, CALAY and JENSEN 1973). CONNEY and BURNS (1972) investigated the metabolic interactions among environmental chemicals and commonly used clinical-drugs. BRODEIUR (1967) reported that phenobarbital increases the tolerance of rats to the toxic effects of malathion and EPN. MENZER and BEST (1968) showed that the toxicity of dicrotophos and phosphamidon was decreased in animals pretreated with phenobarbital. DUBOIS and KINOSHITA (1968) reported that phenobarbital pretreatment of rats and mice decreased the toxicity of 11 organophosphorus insecticides. Recently, LITTERST et al (1975) studied the acute toxicity of substrates of the mixed function oxidase system in normal and phenobarbital pretreated mice. However, there is a paucity of data pertaining to the effect of drug substrates and inhibitors of heme and protein synthesis on the toxicity of insecticides. The present studies were therefore designed to investigate the efrects of pretreatment of inducers such as phenobarbital and 3-methylcholanthrene, drug substrates like aminopyrine and ethylmorphine (type I), acetanilide (typeII), inhibitors of heme synthesis such as nickel chloride and cobalt chloride and an inhibitor of protein synthesis namely cycloheximide on the acute oral toxicity of thiodemeton (o-o-diethyl S-2 (ethylthio) ethyl phosphorodithioate), a commonly used organophosphorus insecticide.

MATERIALS and METHODS

Hindustan Antibiotics strain adults male and female rats and mice were used in the present studies. They were housed 10 per cage in an air conditioned room. The animals were provided with standard laboratory diet and water ad libitum.

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Oral doses of thiodemeton ranging from 1 to 10 mg/kg body weight at 6 different dose levels were used for acute 24 hour LD_{50} determination. The studies were carried out in overnight fasted mice and rats according to the procedure of MILLER and TAINTER (1944).

Pretreatment and mortality studies were done as follows. 1. Pretreatment with inducers: Both male rats and mice were treated with phenobarbital (50 mg/kg body weight) for 3 successive days and 3-methlcholanthrene (15 mg/kg body weight) for 2 days, 2. pretreatment with substrates: ethylmorphine and aminopyrine (type I) and acetanilide (type II) were injected intraperitoneally at the dose level of 40 mg/kg body weight each to both mice and rats for 3 successive days, 3. pretreatment with inhibitors of heme synthesis: rats and mice were injected with nickel chloride and cobalt chloride (5 mg/kg body weight each) for 3 days, 4. pretreatment with an inhibitor of protein synthesis: cycloheximide (0.5 mg/kg body weight) was injected intraperitoneally for 2 days, 5. control group of animlas received an equivalent amount of saline for 3 days or oil for 2 days, 6. thiodemeton administration : animals from groups 1 to 5 were further treated with thiometon at the LD_{85} dose level i.e. 12.3 mg/kg body weight in rats and 9.97 mg/kg body weight in mice respectively. The number of deaths occurred in a period of 24 hours after thiodemeton administration were recorded.

RESULTS

The acute 24 hours LD_{50} values for thiodemeton were found to be 7.2 mg/kg body weight (7.0-7.3, 95% confidence limits) and 5.8 mg/kg body weight (5.6-5.9, 95% confidence limits) in male rats and mice, whereas, in female rats and mice the values were 3.2 mg/kg body weight (3.0-3.3, 95% confidence limits) and 2.7mg/kg body weight (2.5-2.8, 95% confidence limits) respectively.

Table 1 shows the per cent mortality caused by thiodemeton administration to saline, phenobarbital, oil and 3-methylcholanthrene pretreated rats and mice at different time intervals. In the case of saline treated rats, thiodemeton administration resulted in about 16% mortality in first 4 hours which gredually increased up to 10th hour, the mortality being 84%; whereas, in the case of mice, animals started showing death (12%) 2 hours after thiodemeton administration. 27% after 4 hours. 36% after 7 hours and 73% after 11 hours. Animals pretreated with phenobarbital showed 100% protection against the toxicity of thiodemeton both in mice and rats. Rats receiving oil in addition to thiodemeton started showin death (16%) 2 hours after thiodemeton administration, which remained unchanged up to 5th hour and then a gradual increase in the rate of mortality up to 84% was observed in 9 hours duration. Whereas in the case of mice, animals started dying (12%) 3 hours after thiodemeton administration, mortality (36%) increased after 6 hours and was maximum (73%) after 11 hours duration.

TABLE 1

Effect of pretreatment of phenobarbital and 3-methylcholanthrene on thiodemeton induced mortality in rats and mice.

Time in hours	% Mortality							
	Saline		Phenobarbital		Oil		3-Methylcholanthrene	
	Rats	Mice	Rats	Mice	Rats	Mice	Rats	Mice
1	0	0	0	0	0	0	0	0
2	0	12	0	0	16	0	16	0
3	16	12	0	0	16	12	16	0
4	16	27	0	0	16	12	16	0
5	27	27	0	0	27	12	27	12
6	50	27	0	0	36	36	27	12
7	64	36	0	0	50	36	27	12
8	64	36	0	0	64	50	27	12
9	73	50	0	0	84	50	27	27
10	84	50	0	0	84	50	27	27
11	84	73	0	0	84	73	27	36
12 to 24	84	73	0	0	84	73	27	36

TABLE 2

Effect of pretreatment of nickel chloride, cobalt chloride and cyclohexiмide on thiodemeton induced mortality in male rats and mice.

Time in hours	% Mortality							
	Saline		Nickel-Cl_2		$CoCl_2$		Cycloheximide	
	Rats	Mice	Rats	Mice	Rats	Mice	Rats	Mice
1	0	0	0	0	0	0	27	12
2	0	12	16	12	27	0	36	27
3	16	12	27	27	50	12	64	36
4	16	27	27	27	64	27	84	36
5	27	27	36	27	64	27	100	64
6	50	27	50	50	64	36	-	64
7	64	36	73	50	84	50	-	73
8	64	36	73	73	100	50	-	73
9	73	50	100	73	-	73	-	100
10	84	50	-	73	-	88	-	-
11	84	73	-	73	-	88	-	-
12	84	73	-	73	-	100	-	-
13	84	73	-	88	-	-	-	-
14 to 24	84	73	-	88	-	-	-	-

Animals pretreated with 3-methylcholanthrene showed some protection i.e. 73% in both rats and mice against thiodemeton toxicity, within a period of 9 hours.

Pretreatment of animals with ethylmorphine showed 100% mortality due to thiodemeton within a period of 4 hours in rats and 7 hours in mice. (Figure 1). Similarly, rats receiving aminopyrine showed 100% mortality 7 hours after thiodemeton administration; whereas, in the case of mice, death (27%) occured from 3rd hour and reached the maximum i.e. 64%, 9 hours after thiodemeton administration and reached 100% within a period of 11 hours, however, the death (12%) occured in mice 3 hours after thiodemeton administration, remained unchanged upto 8th hour and then increased gradually to the maximum i.e. 64% after 13 hours.

Both rats and mice pretreated with nickel chloride started showing death within a period of 2 hours after thiodemeton administration and reached the maximum i.e. 100% in rats after 9 hours and 88% in mice after 13 hours (Table 2). Similarly, animals pretreated with cobalt chloride showed 27% mortality in rats and 2% in mice after 2 and 3 hours of thiodemeton administration respectively. The cent per cent mortality was observed with a period of 8 hours in rats and 11 hours in mice.

A gradual increase in the rate of mortality i.e. 27,36, 64, 84 and 100% from 1,2,3,4 and 5 hours duration respectively was observed due to thiodemeton administration to rats pretreated with cycloheximide; whereas, in the case of mice, the effect was slightly less i.e. 36% mortality was observed within 3 hours period and thereafter increased to 100% at the end of 8th hour after thiodemeton administration.

DISCUSSION

Several investigators have correlated treatment with inducers or inhibitors with changes in mortality (BIANCHI et al 1973, TALCOTT and STOHS 1973, ALARY and BRODEIUR 1970) in laboratory animals. CUMMINGS and WALTON (1973) showed a positive relation between treatment with known enzyme inducing agents and decreased toxicity of certain chemical carcinogens. Similarly, TUCHWEBER et al (1974) showed a decrease in mortality and liver damage with alkaloids in animals that had been pretreated with enzyme inducers. ALARY and BRODEIUR (1970) showed that the *in vitro* metabolism of the organophosphorus insecticides parathion and paraxon in phenobarbital treated animals closely correlate with the LD_{50} in control vs phenobarbital treated groups. LITTERST et al (1975) studied the reflection of *in vitro* changes in substrate metabolism due to *in vivo* toxicity of mixed function oxidase substrates in phenobarbital pretreated animals. The observed different levels of protection due to phenobarbital and 3-methylcholanthrene, in the present studies could be due to the induction of two different interconvertible forms of cytochrome P-450.

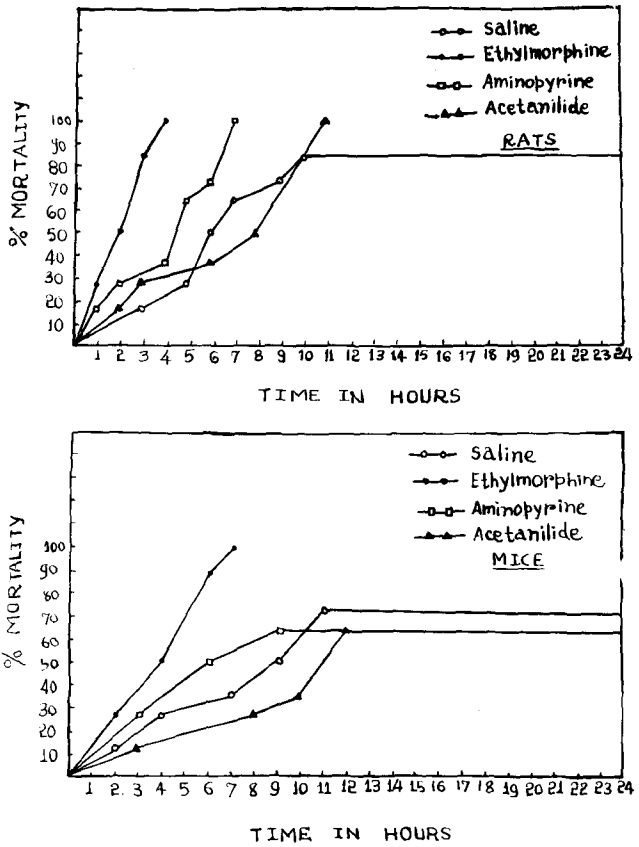

Figure 1. Effect of pretreatment of ethylmorphine, aminopyrine and acetanilide on thiodemeton induced mortality in male rats and mice.

It has been shown that morphine glucuronidation is increased following pretreatment with chloroquine and that this increase in glucuronidation is correlated with the increase in the lethal dose of morphine in mice (SANCHEZ et al 1969). The observed increase in mortality due to thiodemeton administration to ethylmorphine pretreated animals could possibly be due to the in vivo formation of ethylmorphine glucuronide. Our observations are supported by the reports of STOWE and PLAA (1968). Similarly, formation of 4-aminoantipyrine during metabolism of aminopyrine may be responsible for the enhanced toxicity of thiodemeton in

aminopyrine pretreated animals. The enhanced rate of mortality due to thiodemeton administration to animals pretreated with acetanilide remains to be answered. The per cent increase in the toxicity of thiodemeton due to nickel chloride and cobalt chloride as well as cycloheximide pretreatments could be due to the inhibition of heme and protein synthesis respectively.

Futher studies pertaining to the mechanism of action of thiodemeton are in progress.

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