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# THE EFFECTS OF ALTERING THE HEPATIC DRUG METABOLIZING ENZYME ACTIVITY ON THE ACUTE TOXICITY OF DIETHYL-4-NITROPHENYL PHOSPHOROTHIOATE (PARATHION) IN FEMALE RATS[1]

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***ABSTRACT:*** *The effects of altering the hepatic mixed-function oxidase(MFO) activities on the acute toxicity of parathion were examined in female rats. Phenobarbital sodium pretreatment (50 mg/kg/day, i.p.) for 4 consecutive days has resulted in significant decreases in the toxicity of parathion (2 or 4 mg/kg, i.p.) as determined by lethality and cholinesterase activities whereas the toxicity arising from a single dose of $CCl_4$(2 mmol/kg, i.p.) 24 hr prior to parathion challenge was potentiated. These results suggest that the liver plays a more active role in the detoxification of parathion rather than the metabolic activation of this particular insecticide. However, pretreatments with $CoCl_2$ (60 mg/kg/day, sc, for 2 days) or SKF 525-A (50 mg/kg, i.p., 1 hr prior to parathion) did not affect the parameters of parathion's acute toxicity. At the doses of $CCl_4$, $CoCl_2$ and SKF 525-A used in this study, the hepatic p-nitroanisole O-demethylase and aminopyrine N-demethylase activities were inhibited, but the degree of inhibition by each inhibitor did not appear to be different from one another. These results indicate that the altered hepatic MFO activities may not provide the direct underlying mechanism of the protective or potentiation action of parathion's acute toxicity which has been observed with phenobarbital or $CCl_4$ pretreatment, respectively.*

***Keywords:*** *parathion, phenobarbital, carbon tetrachloride, SKF 525-A, $CoCl_2$, acetylcholinesterase activity, mixed-function oxidase (MFO) system.*

## INTRODUCTION

The phosphorothioate insecticides are metabolized by the mixed-function oxidase

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(MFO) enzyme systems to the corresponding oxygen analogs, oxons, which are known to be potent inhibitors of cholinesterase. However, the toxicological significance of phosphorothioate activation is unclear. Several investigators have suggested that the active metabolite formed in the liver is not released into the systemic circulation due to hydrolysis by hepatic esterases (Neal, 1967; Alary and Brodeur, 1969; Sultatos *et al.*, 1985). Although the majority of phosphorothioate activation is expected to occur within the liver, there is much evidence indicating that the extrahepatic activation may be involved in mediating the acute toxicity of these insecticides. In fact, the capacity of extrahepatic tissues such as lungs, kidneys, brain and intestines to activate the phosphorothioate insecticides to the active oxons has been reported in several *in vitro* studies (Poore and Neal, 1972; Law *et al.*, 1974; Norman and Neal, 1976).

Pretreatment of animals with inducers of the hepatic MFO systems such as phenobarbital and chlorinated insecticides has been reported to decrease the acute toxicity of parathion (Triola and Coon, 1966; DuBois and Kinoshita, 1968; Purshottam and Kaveeshwar, 1982; Sultatos, 1986). These authors have attributed the decreases in parathion toxicity to the increased degradation of parathion. They hypothesized that both the increases of metabolic activation and detoxicification of parathion would result in increased degradation of parathion in the liver leading to lower concentrations of parathion and paraoxon at the active sites of toxic action such as brain and lungs. Inherent to this hypothesis is the proposal that any paraoxon formed in the liver is inactivated prior to reaching the lungs and brain.

However, not all studies have been consistent with supposition that the liver plays a net detoxicification role for the phosphorothioate insecticides. Parathion infused into the hepatic portal system resulted in higher lethality than when infused into the general circulation by the way of the femoral vein, suggesting that hepatic activation of parathion contributed significantly to the manifestation of the parathion toxicity (Gaines *et al.*, 1966). Likewise, phenobarbital pretreatment has increased the toxicity of phosphorodithioates activated by the mixed function oxidase systems (Menzer and Best, 1968).

The present study evaluated the role of liver in mediating the acute toxicity of parathion by utilizing compounds known to induce or inhibit the activities of MFO systems. The consequence of hepatic biotransformation of phosphorothioate insecticides has important toxicological implications, that is, if the lungs, not the liver, were to serve as a major site of bioactivation, respiratory exposures might pose more serious risk to the workers than predicted from the results of oral or i.p. exposure studies.

## MATERIALS AND METHODS

### Animals and Treatment

Female Sprague-Dawley rats (100-150 g) were acclimated in environmentally controlled rooms (temperature: 21 ± 1°C, light: 0800-2000, dark: 2000-0800) at least for 10 days prior to experimentation. Lab chow and tap water were allowed *ad libitum*.

Phenobarbital sodium was dissolved in distilled water and administered i.p. at a daily dose of 50 mg/kg for 4 consecutive days. A single dose of $CCl_4$ (2 mmol/kg, i.p.)

was given 24 hr prior to the parathion treatment. Cobaltous chloride (60 mg/kg/day, s.c.) was administered for 2 days. SKF 525-A (50 mg/kg, i.p.) was given 1 hr prior to a challenge dose of parathion. The vehicle used for $CCl_4$ was corn oil whereas distilled water was used for SKF 525-A and $CoCl_2$. Control rats received only the appropriate vehicle. Parathion was dissolved in 20% ethanol and 80% propylene glycol.

## Chemicals

Parathion, purity of 99%, was purchased from Chem Service (West Chester, PA). Acethylthiocholine iodide, DTNB (5,5'-dithio-bis-2-nitrobenzoic acid), cobaltous chloride, NADP, glucose-6-phosphate, NADPH and semicarbazide hydrochloride were purchased from Sigma Chemical Company (St. Louis, MO). Nicotinamide, glycine, zinc sulfate, ammonium acetate, 2,4-pentanedione, barium hydroxide and magnesium chloride were obtained from Aldrich-Chemie GmbH & Co. (Steinheim, West Germany). Other chemicals used in this study include carbon tetrachloride (Junsei Chemical Co., Tokyo, Japan), *p*-nitroanisole (Eastman Kodak Co., Rochester, NY), aminopyrine (Dongwha Pharmaceuticals, Seoul) and phenobarbital sodium (Daiwon Pharmaceuticals, Seoul). SKF 525-A was a kind gift of Smith Kline Beckman Corp. (Philadelphia, PA). All other reagents and solvents were reagent grade or better.

## Enzyme Assays

Cholinesterase activity was determined using a modification of the method of Ellman *et al.* (1961).

Blood was sampled from the orbital sinus of rats using a heparinized microtube and diluted 10X with 0.1M phosphate buffer (pH 7.8). Rats were decapitated and brains and lungs were excised. Tissues were homogenized in 0.1M phosphate buffer (pH 7.8) with a Teflon-glass Potter-Elvehjem homogenizer or a polytron. The tissue homogenates contained 30 mg of brain or 70 mg of lung tissues in a 1.0 m*l* aliquot. Incubation mixture contained 0.1M phosphate buffer (pH 8.0) 3.0 m*l*, DTNB 0.1 m*l* (10 mM), acetylthiocholine iodide 0.02 m*l* (75 mM), and 0.02 m*l* of tissue homogenates or diluted blood. The absorbance at 412 nm was determined using a spectrophotometer. The contents were transferred to a tube and incubated for 30 min at 27°C. The absorbance was again determined, and the initial reading was subtracted. All measurements were made in duplicate. Tissue blanks were established and the absorbance due to spontaneous hydrolysis of the substrate was subtracted from the final measurement.

The MFO activities in the liver, lungs and brain were determined with *p*-nitroanisole and aminopyrine as substrates. *p*-Nitroanisole O-demethylase activity was measured using a modification of the method of Neal and DuBois (1965). A modification of the method of Nash (1953) was used for the determination of aminopyrine N-demethylase activity.

## Statistical Analysis

Results were analyzed by a two-tailed Student's *t*-test. Lethality data were compared using a $X^2$ analysis. In *in vitro* drug metabolism studies, data were analyzed by an analysis of variance followed by Duncan's new multiple range test (steel and Torrie, 1960). The acceptable level of significance was established at P 0.05 except when otherwise indicated.

## RESULTS AND DISCUSSION

Daily treatment of rats with phenobarbital for 4 consecutive days has resulted in decreased lethality with a challenge dose of parathion (Table 1). This observation confirms the results of earlier studies (DuBois and Kinoshita, 1968; Sultatos, 1980; Purshottam and Kaveeshwar, 1982; Sultatos *et al.*, 1984). Also phenobarbital pretreatment antagonized the anticholinesterase activity of parathion suggesting that the protective effect of phenobarbital pretreatment is due to less amount of the active oxygen analog of parathion present at the active sites of its toxic action.

Only a few studies were conducted to determine the effects of metabolic inhibitors on the toxicity of parathion. Massari *et al.* (1969) reported that both parathion activation and paraoxon degradation are delayed in $CCl_4$ treated rats. In the present study, the effects of $CCl_4$ pretreatment on the acute toxicity of parathion were measured. A single dose of $CCl_4$ potentiated significantly the acute toxicity of parathion as indicated by the increased lethality and decreases in AChE/ChE activities (Table 2).

**Table 1.** The effect of phenobarbital on the AChE/ChE activities of rats treated with parathion (2 mg/kg) and on the lethality of a challenge dose of parathion (4 mg/kg).[a]

| Pretreatment | Cholinesterase activity | | Lethality |
|---|---|---|---|
| | Blood (nmol/$\mu$l/min) | Brain (nmol/mg/min) | |
| Control | $0.26 \pm 0.06$[b] | $1.26 \pm 0.09$ | 60% (6/10) |
| Phenobarbital | $0.56 \pm 0.09$ | $2.76 \pm 0.53$[c] | 0% (0/10)[d] |

[a]Rats were pretreated with daily i.p. injection of phenobarbital (50 mg/kg) in distilled water, or distilled water alone, for 4 days prior to the challenge with parathion. Rats were observed for 24 hrs for mortality. AChE/ChE activities were determined 2 hrs following the parathion treatment.

[b]Each value represents the mean ± S.E. for 4 or 5 rats.

[c]Significantly different from the control (Student's *t*-test, $P < 0.05$).

[d]Significantly different from the control ($X^2$ analysis, $P < 0.01$).

The above results support the hypothesis proposed by several investigators that the acute toxicity of phosphorothioates such as parathion is mediated by extrahepatic biotransformation. That is, although much of parathion is metabolized to paraoxon, diethyl phosphorothioic acid and diethyl phosphoric acid in the liver, the paraoxon formed does not escape form the liver due to hydrolysis by various esterases. The concentrations of parathion and paraoxon at the active sites of toxic action, therefore, would be increased by an inhibitor of mixed function oxidase system while the pretreatment with an inducer of the system decrease the parathion/paraoxon concentrations. Increased toxicity of parathion in partial hepatectomized rats also supports this hypothesis (Jacobsen *et al.*, 1973).

In the present study, the effects of using other inhibitors, such as SKF 525-A and $CoCl_2$, were also examined. Neither the lethality nor the AChE/ChE activities were altered by the SKF 525-A pretreatment (Table 3 and 4). Cobaltous chloride treatment enhanced the lethality of parathion, however, the blood ChE activity was significantly

**Table 2.** The effect of $CCl_4$ on the AChE/ChE activities and lethality of rats treated with parathion (2 mg/kg)[a]

| Pretreatment | Cholinesterase activity | | | Lethality |
|---|---|---|---|---|
| | Blood (nmol/μl/min) | Brain (nmol/mg/min) | Lung (nmol/mg/min) | |
| Control | 0.36 ± 0.08[b] | 1.78 ± 0.76 | 0.80 ± 0.08 | 12.5% (1/8) |
| $CCl_4$ | 0.04 ± 0.01[c] | 0.55 ± 0.14 | 0.26 ± 0.09[c] | 75.0% (6/8)[d] |

[a]Rats were pretreated intraperitoneally with a single dose of $CCl_4$ (2 mmol/kg) or vehicle alone 24 hrs prior to the challenge with parathion. Rats were observed for 24 hrs for mortality. AChE/ChE activities were determined 2 hrs following the parathion treatment. For rats which did not survive for 2 hrs, the brains and lungs were excised immediately following death for the measurement of AChE/ChE activities.
[b]Each value represents the mean ± S.E. for 8 rats.
[c]Significantly different from the control (Student's *t*-test, $P<0.05$).
[d]Significantly different from the control ($X^2$analysis, $P<0.05$).

higher than the parathion control. These contradictory observations may be explained by the increased death ratio due to the toxicity of cobaltous ions not related to its effect on metabolism. That is, since more animals died in the early stages of the experiment, relatively insensitive rats to parathion would have been available for the determination of ChE activity in blood. This explanation is supported by the brain and lung AChE activity which was not different from the corresponding parathion control.

The failure of SKF 525-A to affect the AChE/ChE activities and lethality of parathion-treated rats is in line with the report that SKF 525-A did not potentiate the toxicity of parathion, even providing some protection against the toxicity in several fish species (Gibson and Ludke, 1973).

The effects of inducing one or more forms of hepatic cytochrome P-450 on *in vitro* metabolic degration of phosphorothioates such as parathion have been well-documented (Kulkarni and Hodgson, 1980). However, it has been demonstrated that alterations in the toxicity of parathion brought about by pretreatment with an inducing agent do not parallel with the changes in microsomal metabolism of parathion brought about by the same inducing agent (Chapman and Leibman, 1971). Proposals put forth to explain this observation include preferential induction for the detoxicification of parathion *in vivo* (Alary and Brodeur, 1969) as well as the involvement of extrahepatic activation in mediating the phosphorothioate toxicity (Poore and Neal, 1972; Sultatos *et al.*, 1985). The consequences of enzyme induction on the acute toxicity of parathion must be complex since the metabolic activation and detoxicification of this pesticide in the liver are influenced by many factors including the relative rates of activation and detoxicification, the transit times within the liver, and the anatomical localization of enzymes mediating these reactions. Sultatos (1986) observed that phenobarbital pretreatment did not alter the rate of production of paraoxon metabolite from parathion in mouse livers perfused *in situ*, although it increased production of inactive metabolites of parathion. The author concluded that the enhanced detoxicification of parathion to inactive metabolites likely resulted in the protective effects caused by the phenobarbital treatment against parathion's acute toxicity.

It has been reported that cobaltous chloride inhibits the biosynthesis of cytochrome P-450 without affecting the microsomal protein contents (Telphy and Hibbeln, 1971). It has also been widely accepted that SKF 525-A and its metabolite(s) form a stable

**Table 3.** The effect of $CoCl_2$ and SKF 525-A on the inhibition of AChE/ChE activities produced by parathion (2 mg/kg) in rats[a].

| Pretreatment | Blood (nmol/μl/min) | Brain (nmol/mg/min) | Lung (nmol/mg/min) |
|---|---|---|---|
| Control | 0.21 ± 0.04[b] (N = 17)[c] | 2.81 ± 0.67 (N = 19) | 0.52 ± 0.04 (N = 19) |
| SKF 525-A | 0.19 ± 0.05 (N = 19) | 2.96 ± 0.68 (N = 22) | 0.46 ± 0.04 (N = 22) |
| $CoCl_2$ | 0.46 ± 0.06[d] (N = 10) | 3.83 ± 0.75 (N = 19) | 0.50 ± 0.05 (N = 19) |

[a]Rats were pretreated with either $CoCl_2$ 60 mg/kg, sc, 48 and 24 hrs prior to the challenge with parathion or a singlei.p.dose of SKF 525-A 50 mg/kg 1 hr prior to the parathion treatment. Control animals were challenged with parathion without any pretreatment. AChE/ChE activities were determined 2 hrs following the parathion treatment. For rats which did not survive for 2 hrs, the brains and lungs were excised immediately following death for the measurement of enzyme activity.
[b]Each value represents the mean ± S.E.
[c]The number of animals used.
[d]Significantly different from the control (Student's *t*-test, $P<0.05$)

**Table 4.** The effect of $CoCl_2$ or SKF 525-A on the lethality of a challenge dose of parathion (2 mg/kg) in rats[a].

| Pretreatment | Lethality |
|---|---|
| Control | 8% (2/24) |
| SKF 525-A | 14% (4/28) |
| $CoCl_2$ | 44% (12/27)[b] |

[a]Rats were pretreated with either $CoCl_2$ 60 mg/kg, sc, 48 and 24 hrs prior to the challenge with parathion or a single i.p. dose of SKF 525-A (50 mg/kg) 1 hr prior to the parathion treatment. Control animals were challenged with parathion without any pretreatment. Animals were observed for 2 hrs for mortality.
[b]Significantly different from the control ($X^2$ analysis, $P<0.01$).

complex with cytochrome P-450 resulting in inhibition of the microsomal MFO activity. If the induction of hepatic MFO activity were the sole underlying mechanism of protective effects provided by phenobarbital treatment, then inhibition of the enzyme activity by an inhibitor should result in enhancement of the toxicity. In the present study, however, no alteration in the parathion toxicity was observed in SKF 525-A or $CoCl_2$ treated rats.

The reason for the failure of SKF 525-A or $CoCl_2$ to affect the acute toxicity of parathion is unclear. It is possible that the degree of inhibition of the hepatic mixed function oxidase activity caused by SKF 525-A or $CoCl_2$ was not enough to produce marked alterations in the metabolism of parathion. Thus, *in vitro* drug metabolim activity in lever, lungs, and brain from rats pretreated with an inducer or inhibitors of drug metabolizing enzyme system was determined using *p*-nitroanisole or aminopyrine as substrats (Table 5). At the doses used for the experiments measuring lethality and AChE/ChE activity of parathion treatment, the $CoCl_2$, $CCl_4$ or SKF 525-A pretreatments

**Table 5.** The effect of various inducer and inhibitors on hepatic *p*-nitroanisole O-demethylase and aminopyrine N-demethylase activity[a].

| Pretreatment | Number of Rats | *p*-Nitroanisole O-Demethylase Activity ($\mu$g *p*-nitrophenol formed/50 mg liver/15 min) | Aminopyrine N-Demethylase Activity (HCHO nmol formed/50 mg liver/min |
|---|---|---|---|
| Control | 24 | $1.94 \pm 0.10^{c}$ (100)[b] | $2.14 \pm 0.18^{c}$ (100) |
| Phenobarbital (50 mg/kg, ip) | 6 | $5.70 \pm 0.59$ (294) | $3.10 \pm 0.98$ (145) |
| $CoCl_2$ (60 mg/kg, sc) | 6 | $1.54 \pm 0.10^{c}$ (79) | $1.17 \pm 0.02^{d}$ (55) |
| $CCl_4$ (2 mmol/kg, ip) | 6 | $1.01 \pm 0.05^{d}$ (52) | $1.59 \pm 0.04^{d}$ (74) |
| SKF 525-A (50 mg/kg, ip) | 6 | $0.87 \pm 0.04^{d}$ (45) | $1.71 \pm 0.15^{d}$ (80) |

[a]Rats were pretreated with phenobarbital for 4 days, or $CoCl_2$ for 2 days. The final dose was administered 24 hrs prior to sacrifice. A single dose of $CCl_4$ was administered 24 hrs prior to sacrifice. SKF 525-A was given one hour prior to sacrifice. For each treatment group, 6 control rats were treated with the vehicle used for the injection according to the same dosage regimen. For the determination of hepatic metabolic activity, 6 rat livers were pooled into two groups and the results were expressed as mean ± S.E. Each value in the control group represents the mean ± S.E. for eight pools of three rat livers.

[b]Number in parenthesis indicates the percent of the control activity.

[c, d] Values with same superscript are not significantly different from one another (Duncan's new multiple range test, $P<0.05$).

have resulted in decreases of *p*-nitroanisole O-demethylase activity in liver homogenates to 79%, 52% and 45% of the control, respectively. Aminopyrine N-demethylase activity in the 9,000 g fraction of the liver homogenates was decreased by $CoCl_2$, $CCl_4$ or SKF 525-A pretreatments to 55%, 74% and 80% of the control, respectively. Thus, the overall metabolic activity affected by each inhibitor did not likely to have caused the observed differences in the toxicity of parathion. The modifiers of metabolic activity used in the present study including phenobarbital did not alter *p*-nitroanisole or aminopyrine demethylation activity in brain and lungs (data not shown).

As a conclusion, the results suggested that the changes in hepatic drug metabolizing activity may not be the mechanism of protection or potentiation of parathion's toxicity observed with phenobarbital or $CCl_4$ treatment. Rather, some effects of phenobarbital other than those on drug metabolizing activity, for example, the effects on hepatic protein contents, could be responsible for the increased non-specific binding in the liver resulting in lower concentrations of parathion at the extrahepatic active sites.

It should be noted that the changes in metabolic activity of *p*-nitroanisole and aminopyrine may not correctly reflect the alterations in the biotransformation of parathion. To understand the effects of the these metabolic modifiers used in the present study on the activation and detoxicification of parathion, further studies are being conducted in this laborabory.

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