Bull. Environ. Contam. Toxicol. (1987) 39:815–821

Bulletin of Environmental Contamination and Toxicology

Effects of Phenobarbital Pretreatment on the In Vivo Metabolism of Carbaryl in Rats

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Carbaryl (1-naphthyl-N-methylcarbamate, Sevin®) is an insecticide widely used in agriculture. The metabolism of carbaryl in mammalian systems is extensively reviewed by Kuhr and Dorough (1976) and Hayes (1982). Metabolism studies in rats show that carbaryl undergoes hydrolysis, oxidation, and conjugation. The major urinary products are the glucuronide and sulfate conjugates of hydrolyzed or hydroxylated metabolites of carbaryl (Knaak et al., 1965; Sullivan et al., 1972a; and Chin et al., 1979).

Phenobarbital (PB) pretreatment of animals is known to induce the activity of drug-metabolizing enzymes in liver microsomes (Conney, 1967). Studies by Bock et al. (1973, 1979) have shown that PB-treated rats increase the glucuronidation of test substrates, such as chloramphenicol and bilirubin. Previous studies conducted in this laboratory (Knight et al., 1986) showed that incubation of carbaryl with microsomes obtained from livers of untreated or PB-treated rats resulted in little or no oxidative metabolism of the substrate. In addition, no spectral interactions were observed when carbaryl was added to hepatic microsomal suspensions. The present study was carried out to determine the effect of PB pretreatment on the in vivo metabolism of carbaryl in rats.

MATERIALS AND METHODS

Male Sprague-Dawley (CDBR) rats, weighing 120-150 g, were purchased from Charles River Laboratories, Wilmington, MA. 1-Naphthyl-^{14}C-N-methylcarbamate (^{14}C-carbaryl) of specific activity of 58 mci/nmol was obtained from Amersham Corporation, Arlington Heights, IL, and unlabeled carbaryl was purchased from Chem Service, Inc., Westchester, PA. Sodium PB was purchased from J.T.Baker Chemical Co., Phillipsburg, NJ. Glusulase® (type H-2, 113,000 units β-glucuronidase/ml and 4,260 units sulfatase/ml) was purchased from Sigma Chemical Co., St. Louis, MO.

Four rats per group were pretreated intraperitoneally (i.p.) with PB at a dose of 75 mg/kg/day for 5 days. On day 5, ^{14}C-carbaryl

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was administered orally at a dose of 1.64 mg/kg or 16.4 mg/kg, 4 hours after the i.p. dose of PB. Urine samples were collected for 24 hours following administration of the ^{14}C-carbaryl. To develop the metabolic profile, 0.5 to 1.0 ml aliquots of ^{14}C-labeled urine (approximately 500,000 dpm) were chromatographed on a 1.5 x 24 cm column of diethylaminoethyl (DEAE) cellulose according to the method of Knaak *et al.* (1965) as modified by Sullivan *et al.* (1972a). The column was eluted using ammonium formate buffer (pH 6.5). The elution gradients for the column consisted of 0.005 to 0.01, 0.01 to 0.05, and 0.05 to 0.1M ammonium formate. Four ml aliquots of column eluates were collected and 0.5 ml of every fifth fraction was analyzed by scintillation spectrometry.

To confirm the presence of glucuronide and sulfate metabolites of carbaryl, urine obtained from rats dosed with ^{14}C-carbaryl was treated with Glusulase, a hydrolytic enzyme as follows: One ml each of 0.1N ammonium formate buffer (pH 4.5), urine (adjusted to pH 4.5), and Glusulase was mixed and incubated at 37°C for 4 hr in a water bath with constant stirring. After the 4 hr incubation, an additional 1 ml of Glusulase was added and incubated at 37°C for an additional 20 hr. After the 24 hr incubation period, the mixture was adjusted to pH 6.5 and and an aliquot (containing approximately 500,000 dpm) was subjected to DEAE cellulose chromatography. Differences between experimental groups were calculated using Student's t-test with a p value of 0.05 or less as the level of significance.

RESULTS AND DISCUSSION

Approximately 50% of the administered dose of ^{14}C-carbaryl was excreted in the urine in the first 24 hr. This is in good agreement with the excretion results reported by Lechner and Abdel-Rahman (1986). Table 1 shows the quantitative comparison of *in vivo* derived metabolites of carbaryl in untreated and PB-treated rats. Figure 1 illustrates a comparison of the metabolic profiles of ^{14}C-carbaryl metabolites in urine from untreated and PB-treated rats.

The designation of major metabolites of carbaryl in this study was based on the chromatographic characteristics rather than the actual isolation and verification of the metabolites. Qualitatively, eluted peaks A and B are neutrals to the DEAE cellulose anion-exchange system from which no conjugates have been found. Peak C was previously shown to consist primarily of 5,6-dihydro-5,6-dihydroxycarbaryl glucuronide and a minor unidentified metabolite (unknown I) (Sullivan *et al.*, 1972b). Peak D is another unknown metabolite (unknown II). Peak E was identified as hydroxycarbaryl glucuronide and peak F as 1-naphthyl glucuronide. The peaks G and H were shown to be hydroxycarbaryl sulfate and 1-naphthyl sulfate.

The formation of 1-naphthyl glucuronide and 1-naphthyl sulfate indicate that carbaryl underwent hydrolysis followed by conjugation of hydrolytic products to form the sulfate and glucuronide conjugates. Glusulase treatment of urine from rats dosed with

Table 1. *In vivo* derived metabolites of carbaryl in untreated and phenobarbital-treated rats

Peak Identity	Major Component of Peak	1.64 mg carbaryl/kg		16.4 mg carbaryl/kg	
		Control	PB	Control	PB
		% of ^{14}C recovered from the column			
A,B	Neutral fraction	10.9±1.6	9.3±1.8	14.3±2.4	12.9±1.6
C	Dihydrodihydroxycarbaryl glucuronide + unknown I	21.6±0.5	22.3±4.0	27.6±1.0	20.7±3.7
D	Unknown II	3.9±1.9	3.4±0.5	3.4±0.4	2.9±0.3
E	Hydroxycarbaryl glucuronide	8.4±2.7	9.8±1.2	11.3±2.3	8.8±1.9
F	1-Naphthyl glucuronide	42.5±1.4[a]	39.1±2.7[b]	23.4±4.9[a]	21.8±2.9[b]
G	Hydroxycarbaryl sulfate	2.0±0.4	2.7±0.6	2.1±0.7	2.4±0.6
H	1-Naphthyl sulfate	8.6±1.7	11.3±1.8[d]	15.6±1.3[c]	27.4±3.7[c,d]

Each value represents the mean ±S.E. of four rats. Values having the same superscript are signficantly different from each other ($p<0.05$).

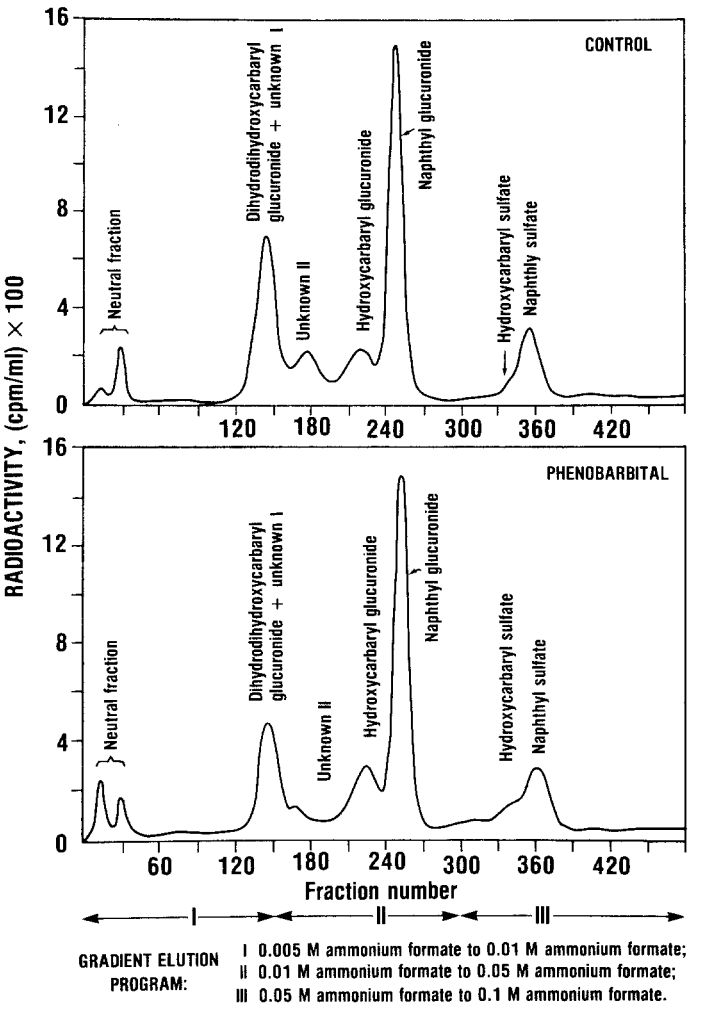

Figure 1. DEAE-cellulose chromatograms of *in vivo* derived metabolites of carbaryl in untreated and phenobarbital-treated rats.

^{14}C-carbaryl confirmed the presence of glucuronide and sulfate metabolites of carbaryl.

Quantitative comparison of *in vivo* derived metabolites of carbaryl in untreated and PB-treated rats are shown in Table 1. In these studies, rats were administered a carbaryl dose of either 16.4 mg/kg or 1.64 mg/kg. The higher dose was such that the rats showed typical signs of acetylcholinesterase inhibition with no mortality. The basis for selection of the lower dose was that the oxidative or conjugative pathways would not be saturated. The quantitative results obtained with untreated rats are in good agreement with the previous report by Knaak *et al.* (1965). When carbaryl was administered at the low dose, 1.64 mg/kg, PB pretreatment did not quantitatively induce either the oxidative or conjugative biotransformation of carbaryl. However, at a 10-fold higher dosage of carbaryl, 16.4 mg/kg, PB pretreatment enhanced only sulfate conjugation of carbaryl. This indicates either that carbaryl may have a higher affinity and accessibility to the cytosolic sulfotransferase or that the glucuronide conjugation pathway may be saturated resulting in a significant increase in sulfate conjugation.

The effect of PB pretreatment on the excretion of total glucuronide and sulfate conjugates of carbaryl in carbaryl-treated rats are shown in Table 2. In PB-treated rats when the carbaryl dose was

Table 2. Effect of phenobarbital pretreatment on the excretion of total glucuronide and sulfate conjugates of carbaryl in carbaryl-treated rats

	1.64 mg carbaryl/kg		16.4 mg carbaryl/kg	
Metabolites	Control	PB	Control	PB
	ug carbaryl equivalent			
Total glucuronide conjugates of carbaryl	29.9±0.8	29.2±0.8	245.7±18.1	234.3±35.2
Total sulfate conjugates of carbaryl	4.4±0.4	5.8±0.5	72.8±4.2[a]	122.0±15.4[a]

Each value represents the mean ±S.E. of four rats.
[a] Values significantly different from each other ($P<0.05$)

increased from 1.64 mg/kg to 16.4 mg/kg an eight-fold increase in urinary excretion of total glucuronide conjugates of carbaryl, and about a twenty-fold increase in excretion of total sulfate conjugates of carbaryl was observed. In confirmation of above data, administration of 16.4 mg carbaryl/kg resulted in a statistically significant ($p<0.05$) increase in urinary excretion of the sulfate conjugates. This would be not only an indication of an apparent saturation of glucuronide conjugation pathway of carbaryl but also an induction of sulfate conjugation pathway of carbaryl. The possibility of PB to induce either the oxidative or the hydrolytic metabolism of carbaryl is not likely since the ratio of total amount of oxidative metabolites of carbaryl to that of hydrolytic metabolites of carbaryl in untreated and PB-treated rats was not appreciably different from each other.

In summary, this study demonstrated that PB pretreatment significantly influenced the metabolism of carbaryl in rats when the insecticide was administered at 16.4 mg/kg but not at 1.64 mg/kg. In the rats treated with the higher dose of carbaryl (16.4 mg/kg), PB pretreatment increased the excretion of sulfate conjugates of carbaryl in urine but failed to induce increased excretion of glucuronide conjugates of carbaryl. These data may be of toxicological importance in humans ingesting drugs which are microsomal enzyme inducers, and who may inadvertently be exposed to small or large amounts of the environmentally-derived pesticide, Sevin®.

Acknowledgments. The authors wish to acknowledge the secretarial assistance of J McWilliams. Supported in part by grant RO 7505 from the Uniformed Services University and by the MITRE Corporation's MITRE Sponsored Research Program.

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Received April 28, 1987; accepted July 28, 1987.