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Effect of Alachlor on the Urinary Excretion of Malondialdehyde, Formaldehyde, Acetaldehyde, and Acetone by Rats

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Alachlor [2-chloro-N-(2,6-diethylphenyl)-N-(methoxymethyl acetamide)] has been widely used as a herbicide for the control of weeds in corn and soybeans (U.S. EPA, 1984). Previous studies have indicated that alachlor can induce DNA damage (Pinter et al., 1989), mutagenicity (Plewa et al., 1984), and nasal tumors in rats (U.S. EPA, 1984). Furthermore, alachlor is cytotoxic to a wide variety of tissues (Rabich et al., 1991; Lin et al., 1987), and it is extensively metabolized in monkeys, rats and mice through several pathways (Sharp, 1988; Feng and Patanella, 1988; Feng et al., 1990; Kimmel et al., 1986). Little information is available regarding the mechanism of toxicity of alachlor.

Recent studies have shown that a wide range of structurally unrelated xenobiotics which induce an oxidative stress enhance the excretion of malondialdehyde (MDA), formaldehyde (FA), acetaldehyde (ACT) and acetone (ACON). For example, carbon tetrachloride, paraquat, endrin, and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) enhance the excretion of these lipid metabolites (Shara et al., 1992).

The detection of urinary lipid metabolites by high pressure liquid chromatography offers a non-invasive and reliable method for assessing lipid metabolism and oxidative stress. We have therefore examined the effect of an orally administered toxic dose of alachlor on the urinary excretion of MDA, FA, ACT, and ACON in rats.

MATERIALS AND METHODS

Alachlor was a generous gift from Monsanto (St. Louis, MO). N-Pentane and acetonitrile were purchased from EM Science, while other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

Female Sprague-Dawley rats, weighing 160-180 g were obtained from Sasco Inc. (Omaha, NE), and housed in stainless steel cages. The animals were kept

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at a room temperature of 20-22°C with a 12 hr light-dark cycle for 3-5 days before use. They were allowed free access to Purina rodent chow and tap water. The rats were given alachlor (800 mg/kg) orally as a single dose in corn oil. The LD_{50} of alachlor administered orally in rats is 1200 mg/kg (Ben-Dyke et al., 1970). The control animals were given the corn oil vehicle.

Rats were placed in metabolic cages (Nalgene Co., Rochester, NY) for urine collection between 6:00 a.m. and 10:30 a.m. The urine collection vessels were positioned over styrofoam containers filled with dry ice which permitted the collection of urine in a frozen state. The mid-point times for the 4.5 hr urine collections occurred at 6, 12, 18, 24, 30, 36, 42 and 48 hrs post-treatment. The same groups of rats were used for the 6 and 30 hr, 12 and 36 hr, 18 and 42 hr, and 24 and 48 hr time points. The animals were allowed free access to water and food except during urine collection, when food was withheld. Four rats were used for each group of treated and control animals.

2,4-Dinitrophenylhydrazine (DNPH) was used as the derivatizing agent in the identification and quantitation of urinary metabolites (Shara et al., 1992). The resulting dinitrophenylhydrazone derivatives were extracted with pentane, and the pentane extracts were evaporated under a stream of nitrogen (Shara et al., 1992). The pentane extracts were reconstituted in acetonitrile, and 20 μL aliquots of each sample were injected onto the HPLC column and eluted isocratically for 40 min. The mobile phase contained acetonitrile/water (49:51) which was filtered (0.45 μ, Nylon-66), degassed using a Millipore filtration kit (Rannin, Woburn, MA) and pumped at a flow rate of 1 ml/min. The detector was set at a wavelength of 330 nm and 0.02 absorbance units full scale (AUFS). The chart recorder speed was 0.25 cm/min. The HPLC system was identical to the system previously described by Shara et al. (1992).

Synthetic hydrazone standards were prepared with formaldehyde (FA), acetaldehyde (ACT), malondialdehyde (MDA) or acetone (ACON) at room temperature. The precipitated hydrazones were filtered, dried and recrystallized from methanol (Shara et al., 1992). Solutions containing 50 ng/μL of the four synthetic hydrazones were prepared and chromatographed as described above. Urine samples were spiked with known amounts of each of the synthetic hydrazones to identify and/or confirm the urinary hydrazones by HPLC co-elution. The percent extraction recoveries of the four standard hydrazones from a pooled urine sample was determined as previously described (Shara et al., 1992).

Calibration curves for each of the synthetic hydrazones were generated by chromatographing 10-700 ng of each and measuring the peak heights. Peak heights were proportional to the amount of hydrazone injected. The data are expressed as nmole/4.5 hrs/kg body weight.

The equipment and the methods used for confirmation of the identities of the lipid metabolites in the urine by gas chromatography-mass spectroscopy were the same as previously described by Shara et al. (1992).

Statistical significance between pairs of mean values from control and treated animals was determined by Student's t test. A $P < 0.05$ was considered significant for all analyses.

RESULTS AND DISCUSSION

The 2,4-dinitrophenyl hydrazone derivatives of the urine samples from control rats exhibited four peaks on HPLC which were identified as malondialdehyde (MDA), formaldehyde (FA), acetaldehyde (ACT) and acetone (ACON) as previously reported (Shara et al., 1992). The identity of these four urinary metabolites was confirmed by gas chromatography-mass spectroscopy as previously described (Shara et al., 1992). The administration of 800 mg alachlor/kg resulted in significant increases in the excretion of these four lipid metabolites. Urine samples were collected up to 48 hrs post-treatment.

Alachlor administration resulted in a 3.5-fold increase in the excretion of formaldehyde (FA) 6 hrs post-treatment, and the excretion of FA increased to approximately 15.5-fold relative to control values 24 hrs after alachlor administration (Table 1). The excretion of FA decreased thereafter, and was approximately 2-fold above control values 48 hrs post-treatment.

The excretion of acetone (ACON) was significantly elevated 6 hrs post-treatment, reaching a maximum excretion of 4.5-fold above control values at 12 hrs post-treatment (Table 1). A biphasic response was observed, with a second increase in excretion occurring at 36 hrs post-treatment, being approximately 2.4-fold above control values. The excretion of ACON returned to control values by 42 hrs post-treatment.

The urinary excretion of acetaldehyde (ACT) is presented in Table 2. An increase of approximately 3.3-fold in the urinary excretion of ACT occurred at 6 and 12 hrs after alachlor administration, and returned to control values thereafter.

The urinary excretion of malondialdehyde (MDA) in response to alachlor is presented in Table 2. Following alachlor administration, the urinary excretion of MDA increased by approximately 2-fold 6 hrs post-treatment and remained relatively constant through the first 36 hrs post-treatment. The excretion of MDA returned to control values 42 hrs after the administration of alachlor.

It is known that many toxicants that induce free radical cell injury enhance the urinary excretion of the four lipid metabolites MDA, FA, ACT and ACON (Comporti, 1989; Stohs, 1990; Numan et al., 1990; Shara, 1992). Alachlor has

Table 1. The urinary excretion of formaldehyde and acetone in the urine of rats treated with alachlor.

Time in hrs after alachlor administration	Excretion of formaldehyde in nmoles/kg body weight/4.5 hrs		Excretion of acetone in nmoles/kg body weight/4.5 hrs	
	Control	Treated	Control	Treated
6	34.01 ± 3.58	121.06 ± 10.43*	2.98 ± 0.31	5.65 ± 0.54*
12	31.56 ± 4.11	278.82 ± 51.52*	3.28 ± 0.71	14.91 ± 2.40*
18	33.07 ± 4.83	298.47 ± 16.59*	3.33 ± 0.69	4.95 ± 0.73*
24	33.78 ± 7.62	524.41 ± 51.39*	2.84 ± 0.58	5.38 ± 1.28*
30	38.10 ± 6.21	283.31 ± 18.25*	3.30 ± 1.16	7.03 ± 0.37*
36	35.95 ± 3.74	290.77 ± 15.58*	3.47 ± 0.55	8.47 ± 0.76*
42	36.85 ± 2.62	131.11 ± 26.01*	3.64 ± 0.40	4.33 ± 0.77
48	35.43 ± 4.83	74.19 ± 8.92*	3.37 ± 0.49	3.10 ± 0.31

Rats were treated with 800 mg alachlor/kg or the vehicle p.o. Each value is the mean ± S.D. from four animals. Each time point represents the midpoint of 4.5 hr urine collection times. *$P < 0.05$ with respect to the corresponding control group.

been shown to be cytotoxic to many tissues (Rabich et al., 1991; Lin et al., 1987). It would therefore be expected to induce lipid peroxidation with subsequent increases in the urinary excretion of lipid metabolites. The excretion of the MDA and ACON tended to be biphasic with peaks at approximately 12 hrs and 36 hrs post-treatment. Larsen and Bakke (1981, 1983) have shown that propachlor, a chloroacetanilide herbicide structurally related to alachlor is metabolized through enterohepatic circulation. Feng et al. (1990) have suggested that enterohepatic circulation is a predominant pathway of alachlor metabolism in rats and mice. In addition, alachlor is extensively metabolized in rats (Brown et al., 1988), and one or more of these

Table 2. The urinary excretion of acetaldehyde and malondialdehyde in the urine of rats treated with alachlor.

Time in hrs after alachlor administration	Excretion of acetaldehyde in nmoles/kg body weight/4.5 hrs		Excretion of malondialdehyde in nmoles/kg body weight/4.5 hrs	
	Control	Treated	Control	Treated
6	1.98 ± 0.28	6.93 ± 0.40*	1.75 ± 0.44	3.47 ± 0.87*
12	2.34 ± 0.47	7.60 ± 1.77*	1.86 ± 0.71	3.34 ± 0.54*
18	2.38 ± 0.74	2.75 ± 0.78	1.77 ± 0.98	3.43 ± 0.25*
24	2.74 ± 0.64	3.06 ± 0.47	2.18 ± 0.49	2.62 ± 0.20*
30	2.57 ± 0.25	3.00 ± 0.94	1.53 ± 0.34	2.78 ± 0.46*
36	2.76 ± 0.55	3.72 ± 0.67	2.35 ± 0.61	4.75 ± 1.48*
42	2.49 ± 0.29	2.03 ± 0.20	2.16 ± 0.52	2.36 ± 0.64
48	2.79 ± 0.68	2.80 ± 0.40	2.05 ± 0.49	2.28 ± 0.19

Rats were treated with 800 mg alachlor/kg or the vehicle p.o. Each value is the mean ± S.D. from four animals. Each time point represents the midpoint of 4.5 hr urine collection times. *$P < 0.05$ with respect to the corresponding control group.

metabolites may induce lipid peroxidation, producing a secondary increase in the urinary excretion of the lipid metabolites. Thus, enterohepatic recycling of alachlor and/or the production of reactive metabolites of alachlor may account for the biphasic excretion of the four lipid metabolites.

Many xenobiotics have been shown to increase the urinary excretion of MDA (Draper et al., 1984). For example, increased excretion of MDA was reported after treatment of rats with paraquat (Tomita et al., 1990), endrin or carbon tetrachloride

(Shara et al., 1992), and adriamycin (Dhanakoti and Draper, 1987). Adriamycin and paraquat are known to undergo redox cycling, while carbon tetrachloride undergoes metabolic activation.

The excretion of acetone in urine is increased by xenobiotics which produce an oxidative stress, including paraquat, endrin, carbon tetrachloride and 2,3,7,8-tetrachlorodibenzo-p-dioxin (Shara et al., 1992). The source of the acetone may be beta oxidation or enhanced lipid peroxidation. However, following alachlor administration it is possible that at least part of the acetone may result from the metabolism of alachlor as suggested by Feng et al. (1990).

The source of the early increase in the excretion of acetaldehyde is not clear. If it arose from the subsequent metabolism of malondialdehyde, a more prolonged increase in its excretion would be expected. The increase in the excretion of acetaldehyde might arise in part from the metabolism of alachlor (Feng et al., 1990).

The most pronounced effect of alachlor on the urinary excretion of the four metabolites was observed with FA. Large increases in the excretion of FA have been observed in response to the administration of other xenobiotics, including, paraquat, TCDD and endrin (Shara et al., 1992). Thus, the urinary excretion of FA appears to be increased by a wide range of xenobiotics. In addition, FA has been reported as a metabolite of alachlor (Brown et al., 1988).

The results clearly demonstrate that the administration of alachlor to rats results in significant increases in the urinary excretion of FA, ACON, ACT, and MDA. The increase in excretion of these urinary products may be due to a combination of increased lipid metabolism and peroxidation, as well as the metabolism of alachlor.

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