THE ROLE OF ENZYME INDUCTION ON METABOLITE FORMATION OF BIS(2-METHOXYETHYL) ETHER IN THE RAT

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The effect of enzyme induction on the metabolism of the reproductive toxicant bis (2-methoxyethyl) ether (diglyme) was studied in male Sprague-Dawley rats. Rats were given either daily doses of diglyme at 5.1 mmol/kg body wt. by gavage or 0.1% (w/v) phenobarbital (PB) in the drinking water for 22 consecutive days. In one study, a significant reduction in the hexobarbital sleeping time was determined for rats pretreated with diglyme or PB in comparison with that determined for naive rats. In a second study, naive and pretreated rats given single oral doses of ^{14}C-diglyme at 5.1 mmol/kg body wt. showed similar urinary ^{14}C excretion patterns. Urinary metabolites were separated and quantified by hplc to evaluate the influence of pretreatment with either diglyme or PB on the ^{14}C-diglyme urinary metabolite profile. The amount of (2-methoxyethoxy) acetic acid, the principal metabolite, was similar for rats given no pretreatment and for rats pretreated with either diglyme or PB. However, both pretreatments resulted in significant increases in the formation of methoxyacetic acid, a recognized reproductive toxicant.

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3. Key words: bis(2-methoxyethyl) ether, glycol ether metabolism, induction.

4. Abbreviations: bis(2-methoxyethyl) ether = diglyme; methoxyacetic acid = MA; phenobarbital = PB; Hexobarbital = HB.

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INTRODUCTION

Bis (2-methoxyethyl) ether [diglyme; CAS No. 111-96-6], is an aprotic glycol ether used in industrial processes as a solvent and as a reduction medium for chemical syntheses. The production of all gylcol ethers has been estimated to exceed 7 million pounds in 1977 (NIOSH, 1983), and both dermal and respiratory exposures of workers have been reported (Zavon, 1963). Recent studies have demonstrated that diglyme causes adverse reproductive effects in rats (McGregor et al., 1983; Cheever et al., 1988b) and mice (Plasterer et al., 1985; Hardin and Eisenmann, 1987; Price et al., 1987). Recent biotransformation studies by Cheever et al. (1988a) have shown that these effects can be attributed to the formation of methoxyacetic acid (MA). The biocleavage of such alkoxyethers is thought to be mediated by an enzymatic O-dealkylase system (Soodsma et al., 1970; Cheever et al., 1984), and it has been suggested that such O-dealkylase activity may be cytochrome P-450 related (Lubet et al., 1985; Guengerich et al., 1985). It has long been known that exposure to chemical xenobiotics can affect metabolism in vivo (Conney et al., 1960) and that such adaptive changes appear to be mediated, in part, by induction of cytochrome P-450 (Alvares et al., 1973). The biological efficacies of such xenobiotics may be dependent on the particular form of cytochrome P-450 induced (Lubet et al., 1985).

Possible chemical interactions resulting from long-term exposure to diglyme itself or to known cytochrome P-450 inducers may result in increased diglyme toxicity. This paper examines the effect of such compounds on diglyme central ether cleavage, the biotransformation pathway reported to be responsible for diglyme toxicity.

MATERIALS AND METHODS

Chemicals. The test compound diglyme (> 99.5%) was purchased from Fluka Chemical Corporation, Happague, NY, and [1,2-ethylene-^{14}C] diglyme was synthesized by Pathfinder Laboratories, Inc., St. Louis, MO, with a specific activity of 0.88 mCi/mmol and a radiochemical purity > 99% as determined by high performance liquid chromatography (hplc). Phenobarbital sodium (PB) and hexobarbital sodium (HB) (> 99%) were purchased from Sigma Chemical Co., St. Louis, MO.

Animals. Male Sprague-Dawley (Crl:CD (SD) BR outbred) rats, weighing 51 to 75 g, were obtained from the Charles River Breeding Laboratories, Inc., Wilmington, MA, and maintained on NIH-07 rat and mouse diet (Ziegler Brothers, Inc., Gardners, PA). The rats were housed 5 per cage, and a 12-hr light/darkness cycle as well as 22-25° C laboratory temperatures were maintained throughout the course of the study. The animals were supplied with food and distilled water, with or without 0.1% PB, ad libitum.

Dosing and Sample Collection. For the sleeping time determinations, a dosing solution of HB was prepared in distilled water at 25 mg/mL. Naive rats or rats

pretreated with either 5.1 mmol diglyme/kg body wt. or 0.1% PB in drinking water, concentrations used in previous metabolism studies (Cheever et al., 1984, 1988a), for 22 consecutive days were given ip injections of the HB at 125 mg/kg body wt. The hexobarbital sleeping times were performed as described by Conney et al. (1960). For the metabolism study, a dosing solution of [1,2-ethylene-^{14}C] diglyme was prepared by dissolving the radiochemical along with non-radiolabeled diglyme in distilled water (1.02 mmol/mL, specific activity = 29 μCi/mmol). The [1,2-ethylene-^{14}C] diglyme dosing solution was administered at 5 mL/kg body wt. at a dose level designed to allow comparison with previous toxicity studies (Cheever et al., 1988). Rats were pretreated with either 5.1 mmol diglyme/kg body wt. or 0.1% PB in drinking water for 22 consecutive days prior to the administration of a single po dose of ^{14}C-diglyme at 5.1 mmol/kg body wt. The dosing solution was administered to naive or pretreated 48-day-old rats by gavage between 8 and 9 a.m., and the animals were immediately placed in separate glass Roth-style metabolism cages. Urine was collected at 6, 12, 24, 48, 72, and 96 hr following the administration of ^{14}C-diglyme for the evaluation of urinary metabolites as described previously (Cheever et al., 1984).

Instrumental Analysis. Prior to analysis by hplc, urine samples were passed through a 0.2-μm pore size filter (Acrodisc,® Gelman Sciences, Ann Arbor, MI). The separation of urinary metabolites was accomplished using a Waters model ALC/GPC 201 chromatographic system equipped with a model 721 system controller, two model 6000A pumps, a WISP 710B sampler, and a model TCM temperature control module (Waters Associates, Inc., Milford, MA). An appropriate aliquot of the filtered urine was injected directly onto a 50-cm-long × 9-mm-i.d. stainless-steel column packed with 10-μm Partisil-OD-2 (Whatman, Inc., Clifton, NJ) in series with a 15-cm-long × 3.9-mm-i.d. stainless-steel column packed with 5-μm spherical NOVA-PAK C18 (Waters Associates, Inc.) and eluted at 1 mL/min with 1% acetic acid in water for 15 min followed by a methanol:1% acetic acid linear gradient from 15% to 80% methanol over a 40-min period. Radiolabeled urinary components were detected and quantified by a Tri-Carb® RAM 7500 radioactivity monitor (Packard Instrument Co., Inc., Downers Grove, IL).

Total radioactivity of the urine was quantified using a Beckman model LS 8100 liquid scintillation spectrometer as described previously (Cheever, et al., 1988a).

Statistical Analysis. Statistical differences between group means were determined using one-way analysis of variance (ANOVA). The level of significance chosen was $P < 0.05$. When ANOVA revealed significant differences between groups, then Dùnnett's test was used to compare treated groups to the vehicle control group (Dunnett, 1964).

RESULTS

The effects of pretreatment of rats with diglyme or PB on both hexobarbital sleeping

time and diglyme metabolism were evaluated. The mean sleeping time was reduced from 28.2 ± 3.7 min for naive rats to 17.8 ± 1.0 and 8.7 ± 0.7 min (Mean ± S.E., n = 10), respectively, for rats pretreated with diglyme or PB, an indication of enzyme induction. The urinary ^{14}C excretion following the administration of radiolabeled diglyme was not significantly affected by the 22-day pretreatment with either diglyme or PB. The 96-hr totals for urinary ^{14}C were 83.4 ± 3.3%, 88.8 ± 0.5%, and 88.9 ± 0.5%, (Mean ± S.E., n = 5), respectively, for rats given no pretreatment or after pretreatment with diglyme or PB. The 96-hr cumulative amounts of radiolabeled urinary components are compared in Table 1. The amount of the major metabolite, (2-methoxyethoxy) acetic acid, was essentially unchanged by pretreatment with either diglyme or PB. Significant increases in the amount of MA, the metabolite associated with the reported toxicity of diglyme, were noted after pretreatment with either diglyme or PB (Figure 1).

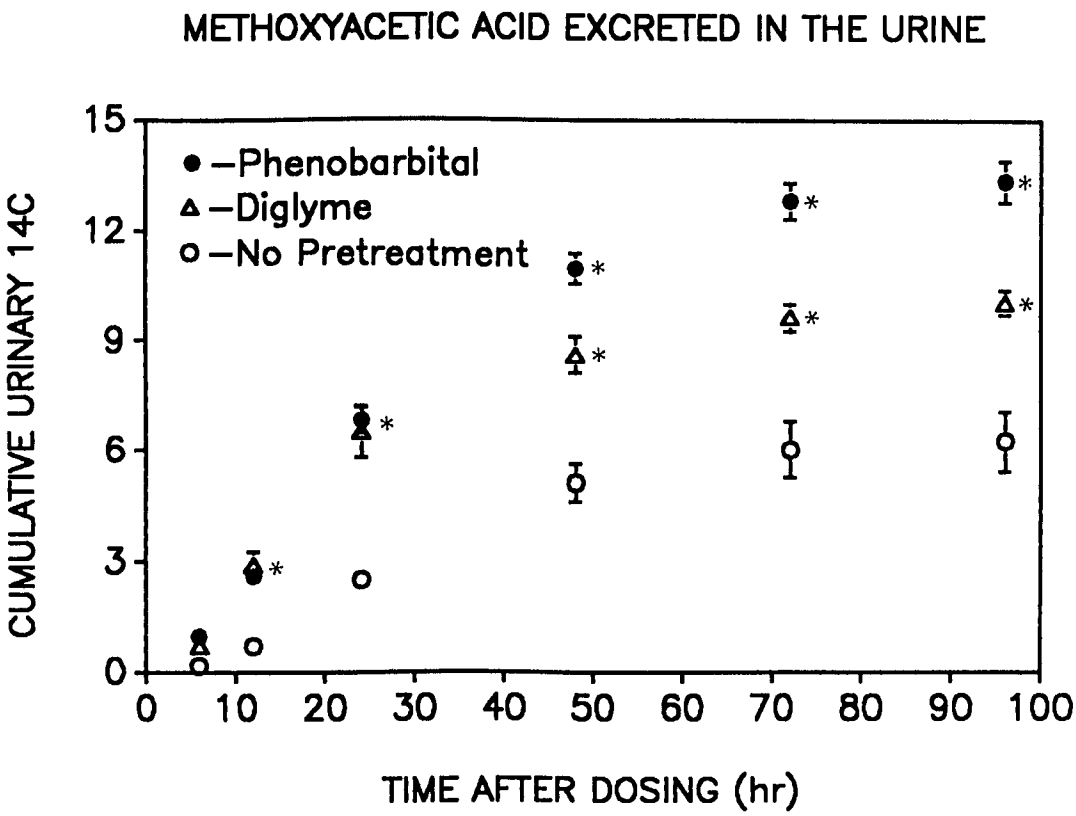

FIGURE 1. Cumulative excretion of methoxyacetic acid from male rats following administration of [1,2-ethylene-^{14}C] diglyme (148 μCi/kg) at 5.1 mmol/kg bw by gavage. The rats, either unpretreated (β) or pretreated with diglyme at 5.1 mmol/kg bw (o) or with 1% phenobarbital in the drinking water (●) for 22 consecutive days, were 48 days old at administration of the radiolabeled compound. Each point represents the Mean ± S.E. for five rats. *Significantly greater than control value ($p < 0.05$).

TABLE 1
Comparison of ^{14}C-Diglyme 96-Hr Urinary Components after 22-Day Phenobarbital or Diglyme Pretreatment[a]

^{14}C Urinary Component[b]	Pretreatment		
	None	Diglyme	Phenobarbital
Metabolite I	0.3 ± 0.1	0.4 ± 0.3	0.7 ± 0.5
N-(Methoxyacetyl)Glycine	0.3 ± 0.2	0.7 ± 0.3	0.9 ± 0.4
Diglycolic acid	3.9 ± 1.0	2.2 ± 0.2	4.6 ± 0.9
Metabolite IV	1.0 ± 0.3	1.1 ± 0.2	1.6 ± 0.3
Methoxyacetic acid	6.2 ± 0.8	10.0 ± 0.2[c]	13.4 ± 0.6[c]
2-Methoxyethanol	0.8 ± 0.3	2.1 ± 0.5	1.5 ± 0.4
(2-Methoxyethoxy)Acetic acid	67.9 ± 3.3	68.5 ± 0.9	64.2 ± 1.6
Metabolite VIII	1.2 ± 0.3	2.3 ± 0.7	1.0 ± 0.5
2-(2-Methoxyethoxy)Ethanol	<0.1	1.2 ± 0.4[c]	0.7 ± 0.6
Diglyme	1.8 ± 0.3	0.3 ± 0.2[c]	0.3 ± 0.1[c]
Total Urinary ^{14}C	83.4 ± 3.3	88.8 ± 0.5	88.9 ± 0.5

[a]Male Sprague-Dawley rats (51.75g) were given either distilled water, phenobarbital (0.1% in drinking water), or diglyme (5.1 mmol/kg bw/day by gavage) for 22 days prior to administration of 5.1 mmol/kg bw (148 μCi/kg) of [1,2-*ethylene*-^{14}C]diglyme by gavage.

[b]Values represent the cumulative amount of metabolites as percent of the administered dose appearing in the urine during the 96-hr sampling period (Mean ± S.E., n = 5). Three urinary metabolites—I, IV, and VIII-were unidentified.

[c]Statistically significant by one-way ANOVA comparison with corresponding rats given no pretreatment ($P < 0.05$).

DISCUSSION

The results of this study indicate that pretreatment of rats with diglyme itself or with PB, a classic inducer of cytochrome P-450 activity, increases the extent of cleavage of the central ether bond in diglyme. Such cleavage leads to the formation of 2-methoxyethanol, the precursor of MA, a compound reported to be a potent reproductive toxicant in the rat. Price and her coworkers (1987) reported significant dose-related postimplantation losses and developmental defects in fetuses after administration of ≥ 250 mg/kg/day of diglyme to the dam. The patterns of fetal malformation observed by these investigators were strikingly similar to those of 2-methoxyethanol (Nagano et al., 1981) and MA (Brown et al., 1984). an observation confirmed by Hardin and Eisenmann (1987). Additionally, tubular atrophy of the testis, resulting from effects of diglyme on the pachytene and dividing spermatocytes (Cheever et al., 1988a) was related to the amount of MA formed. Thus, the possibility of altered metabolism and toxicity, resulting from a shift from a major to a minor pathway, may be an important consideration for risk assessment of occupational exposure to diglyme and related aprotic glycol ether compounds.

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