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A MECHANISTIC STUDY OF THE METABOLISM OF 1,1-DICHLORO-2,2-BIS(*p*-CHLOROPHENYL)ETHANE (DDD) TO 2,2-BIS(*p*-CHLOROPHENYL)ACETIC ACID (DDA)

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Abstract—The metabolism of [1-^{2}H]-1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane (DDD-d) and [1-^{2}H]-1-chloro-2,2-bis(*p*-chlorophenyl)ethene (DDMU-d) and their corresponding non-deuterated isomers DDD and DDMU was studied in female Swiss mice over a 96-hr period. The only detected urinary metabolite of DDD-d and DDD was 2,2-bis(*p*-chlorophenyl)acetic acid (DDA). Animals adminstered DDD excreted ~2.4-fold more DDA than those treated with DDD-d over the total collection period. The initial (0–36 hr) linear excretion rates of DDA for DDD and DDD-d were 17.1 and 5.5 μg/hr respectively. DDMU- and DDMU-d-treated mice excreted significant quantities of DDA, 2,2-bis(*p*-chlorophenyl)ethanol (DDOH) and 2,2-bis(*p*-chlorophenyl)ethanal (DDCHO). The only quantitative difference between DDMU and DDMU-d was that the non-deuterated isomer afforded ~1.8 times more DDA over the 96-hr collection. The initial (0–36 hr) linear excretion rates of DDMU and DDMU-d were 10.7 and 6.2 μg/hr respectively. The qualitative and quantitative results are consistent with DDD being metabolized to DDA via enzyme-mediated hydroxylation on the C-1 side chain carbon.

It has been proposed that the metabolism of DDT† to DDA involves a sequential combination of reductive dechlorination, dehydrochlorination, reduction, hydroxylation and oxidation steps (Fig. 1) [1–5]. In a recent paper, we suggested that the formation of DDA results from the hydroxylation of DDD at C-1, yielding an intermediate that spontaneously loses HCl to afford Cl-DDA, the acid chloride derivative of DDA (Fig. 2) [6]. The basis for this hypothesis is that animals administered DDD excrete only trace amounts of DDOH, but larger quantities of DDA than do animals treated with any other lipophilic metabolite of DDT [6, 7]. The metabolism of DDT shows the same trend, although the quantity of excreted DDA is less. In contrast, DDMU and DDMS yield DDA and DDOH in a ~1:2 ratio [7]. We present here metabolic studies using DDD-d and DDMU-d that provide additional confirmation of the proposed direct DDD to DDA pathway.

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† Abbreviations: DDT, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane; DDA, 2,2-bis(*p*-chlorophenyl)acetic acid; DDD, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane; DDD-d, [1-^{2}H]-1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane; Cl-DDA, 2,2-bis(*p*-chlorophenyl)acetyl chloride; DDMU, 1-chloro-2,2-bis(*p*-chlorophenyl)ethene; DDMU-d, [1-^{2}H]-1-chloro-2,2-bis(*p*-chlorophenyl)ethene; DDMS, 1-chloro-2,2-bis(*p*-chlorophenyl)ethane; DDOH, 2,2-bis(*p*-chlorophenyl)ethanol; DDCHO, 2,2-bis(*p*-chlorophenyl) ethanal; and DDA-methyl ester, methyl 2,2-bis(*p*-chlorophenyl)acetate.

MATERIALS AND METHODS

Materials

DDOH, DDD and DDA were purchased from the Aldrich Chemical Co., Milwaukee, WI, and DDMU and DDCHO were prepared as described previously [7]. Female Swiss mice (Eppley colony) weighing ~35 g were used for all metabolism studies.

Chemicals

Preparation of DDD-d. DDT (3 g, 8.5 mmoles), mercuric chloride (0.3 g, 1.1 mmoles), aluminium foil (1.6 g, 59.3 mmoles), methanol-d_4 (50 ml, 99.5 atom%), and benzene (80 ml) were refluxed for 18 hr and filtered, and the filtrate was concentrated *in vacuo*. The residue was crystallized from hexane to yield 2.32 g (77%) of white solid, which was homogeneous by GLC (10% SP2100; column temp. 230°) and contained 99.3 atom% d(GLC—MS *m/z* 319, 321 and 323 peak intensities relative to authentic non-deuterated material).^{1}H-NMR also indicated complete deuteration of DDD.

Preparation of DDMU-d. DDD-d (1.5 g, 4.7 mmoles) potassium hydroxide-d (4 g, 70.1 mmoles), and methanol-d_4 (40 ml) were refluxed for 16 hr. H_2O (50 ml) was added, and the mixture was extracted with methylene chloride (2 × 100 ml). The organic layer was dried and concentrated *in vacuo*, and the residue was purified by preparative TLC (2 mm silica gel, hexane solvent) and crystallization from hexane to afford 0.72 g (54%) of white solid, which was homogeneous by GLC (10% SP-2100, column temp. 230°) and HPLC (4.6 mm × 25 cm Ultrasphere ODS column,

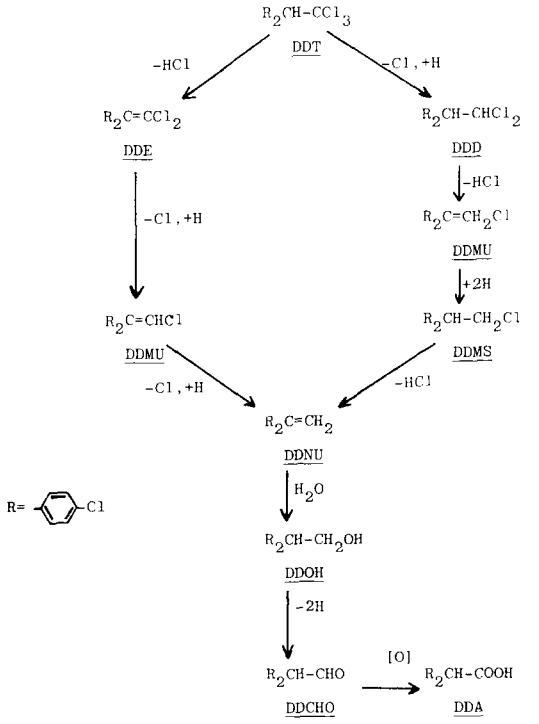

Fig. 1. Sequential scheme for the metabolic disposition of DDT in solvents.

5 μm; solvent, 9% H_2O/91% MeOH; flow rate 1 ml/min; detection, 238 nm). The product contained 99.2 atom% d determined by GLC–MS (*m/z* 283, 285 and 287 peak intensities relative to authentic non-deuterated material). ^{1}H-NMR also showed complete deuteration of DDMU.

Animal treatment

A dose of 250 mg/kg of the appropriate compound in 0.15 ml olive oil was administered by gavage, and the mice were placed in glass metabolism cages (one pair/cage) that allowed separate collection of urine and feces. The collectors of the cages were submerged in solid CO_2 to freeze urine as it was excreted. Collectors were changed every 12 hr for a total analysis period of 96 hr.

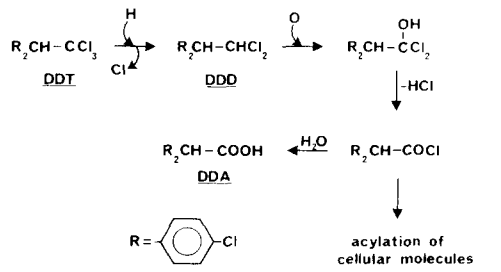

Fig. 2. Scheme for the direct conversion of DDD to DDA.

Metabolite analysis

The urine samples were prepared for analysis by previously reported procedures [6, 7], which allowed DDA to be analyzed as the corresponding methyl ester. The yields of DDA-methyl ester and DDOH have been corrected for recovery [7], while all other metabolite yields are uncorrected. GLC analysis and quantitation were carried out as previously described [6, 7].

P-450 analysis

DDD, DDD-d, DDMU, or DDMU-d was administered to mice, by gavage, at a dose of 250 mg/kg in 0.15 ml olive oil (see Table 2). Control animals received only 0.15 ml olive oil. Animals were killed at time intervals of 24, 48, 72 and 96 hr after treatment. Their livers were then excised, and hepatic P-450 levels were determined [8]. Protein quantitation was done according to Lowry *et al.* [9].

RESULTS

The urinary metabolites of deuterated and non-deuterated DDD and DDMU over the 96-hr collection period are presented in Table 1. With an ~5 μg threshold of detection, the only metabolite observed from DDD-treated mice was DDA. The difference in excreted DDA from DDD-d and DDD was significant, with the latter compound yielding ~2.4-fold more DDA. DDMU and its deuterated analogue yielded significant amounts of DDA, DDOH and DDCHO. The only difference between the deuterated and non-deuterated compound was

Table 1. Urinary metabolites of deuterated and non-deuterated DDD and DDMU

Administered[a] compound	Metabolites (μg)[b]		
	DDA[c]	DDOH	DDCHO
DDD	915 ± 101	ND[d]	ND
DDD-d	384 ± 20	ND	ND
DDMU	572 ± 93	329 ± 17	58 ± 16
DDMU-d	316 ± 62	399 ± 127	77 ± 30

[a] The compounds were administered by gavage in 0.15 ml of olive oil using a dose of 250 mg/kg body weight.

[b] The levels reported are the means from at least 5 pairs of mice from 4 days of urine collection (± S.D.).

[c] DDA was derivatized to and quantitated as its methyl ester.

[d] ND = not detected; limit of detection 5 μg.

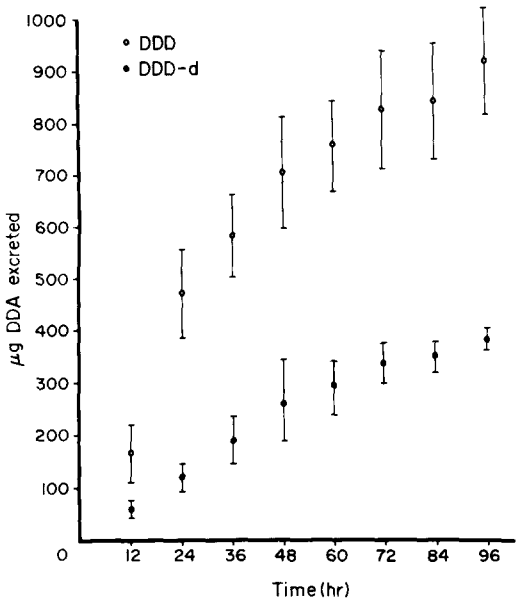

Fig. 3. Rate of DDA excretion after administration of DDD and DDD-d. Values reported are means ± S.D.

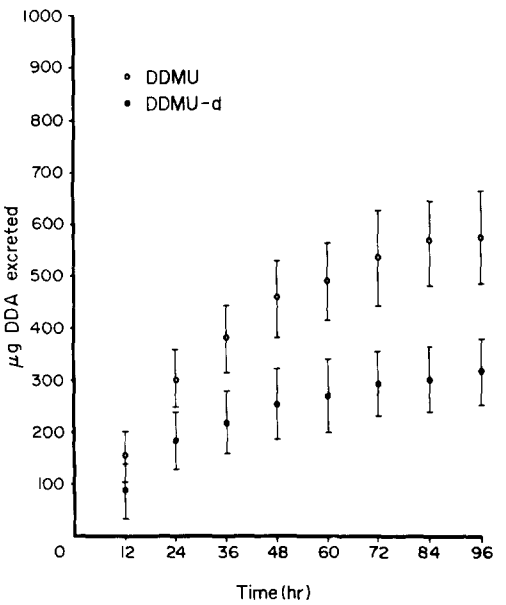

Fig. 4. Rate of DDA excretion after administration of DDMU and DDMU-d. Values reported are means ± S.D.

that the latter afforded ~1.8 times more DDA. The rate of DDA excretion from DDD-d and DDD is shown in Fig. 3. The excretion rate for the deuterated compound in the first 36 hr was 5.5 μg/hr, as compared to 17.1 μg/hr for the non-deuterated compound. The initial rates for both DDD-d and DDD were linear (correlation coefficients = 0.99). A similar rate plot for DDMU-d and DDMU appears in Fig. 4. The initial rates (0–36 hr) for the deuterated and non-deuterated compounds were 6.2 and 10.7 μg DDA/hr, respectively, and these were also linear with correlation coefficients of 0.98.

Hepatic P-450 activity was assayed 24, 48, 72 and 96 hr after administration of deuterated and non-deuterated DDD and DDMU. Control animals received only olive oil solvent. No significant change in P-450 levels was observed over the 4-day period between deuterated and non-deuterated treated animals (Table 2).

DISCUSSION

It was originally proposed that the metabolism of DDT in rodents follows the sequential multi-step pathway shown in Fig. 1. The accuracy of this pathway has been challenged in a recent paper, based on metabolic studies of DDT, DDD, DDMU and DDMS in mice and hamsters [6, 10]. In the present paper we proposed an alternative metabolic scheme suggesting that DDA formation results from direct hydroxylation of the chlorinated side chain 1-carbon of DDD to afford an intermediate acid chloride (Fig. 2). Implicit in Fig. 2 is that cleavage of the C–H bond is rate-limiting and, therefore, that replacement of hydrogen with deuterium should elicit a kinetic isotope effect. *In vitro* and *in vivo* isotope studies have demonstrated that C–H bond cleavage is the rate-limiting step in the oxidative metabolism of $CHCl_3$ to phosgene [11–13]. In those studies deuteration

Table 2. Effect of DDD, DDD-d, DDMU and DDMU-d on cytochrome P-450 in mouse liver*

Day#	cytochrome P-450 (nmoles/mg protein)†				
	control	DDMU	DDMU-d	DDD	DDD-d
1	1.13	0.91	0.88	0.71	0.87
2	0.75	1.05	1.28	1.13	1.19
3	0.71	0.96	1.11	0.92	1.06
4	0.77	1.00	0.92	0.82	0.83
4-day average ± S.D.	0.84±0.19	0.98±0.06	1.04±0.18	0.90±0.18	0.98±0.17

* Compounds (250 mg/kg) adminstered by gavage in olive oil. Control animals received only olive oil.
† Values reported were obtained by pooling the livers of 3 mice.
# Time after administration of compounds.

resulted in an approximately 2-fold decrease in phosgene formation. As shown in Table 1 and Fig. 3, the deuteration at C-1 of DDD significantly affected the yield of acid over the 96-hr collection period and, as important, reduced the initial (0–36 hr) rate of DDA excretion to <33% of the non-deuterated compound. The magnitude of this isotope effect is strong evidence that C–H bond cleavage is the rate-determining step in DDA excretion and is consistent with the metabolic pathway proposed in Fig. 2.

For control purposes, the rates of DDA excretion from DDMU-d- and DDMU-treated mice were determined, since DDMU cannot afford DDA by the direct hydroxylation mechanism. From previous studies, we have concluded that conversion of DDMU to DDA involves DDOH and DDCHO as metabolic intermediates [7]. The oxidative conversion of DDMU to DDA must also eventually involve C–H bond cleavage and, therefore, exhibit an isotope effect. However, because there are five metabolic steps in the conversion of DDMU to DDA, the C–H bond cleavage is not anticipated to be the sole rate-limiting process. As seen in Table 1 and Fig. 4, the metabolic formation of DDOH and DDCHO was not affected by the isotopic substitution, while DDA excretion was reduced by about one-half. This is a rather small difference which is consistent with the sequential pathway in Fig. 1. The fact that significant amounts of DDOH and DDCHO were observed in the urine of DDMU- and DDMU-d-treated animals and that the levels of these two polar metabolites were not affected by deuteration is evidence that DDMU and DDD afford DDA via different mechanisms.

Finally, the possibility that deuterated and non-deuterated DDD might have different enzyme-inducing activities was explored by analyzing cytochrome P-450 levels after pretreatment with deuterated and non-deuterated DDD. No significant difference in P-450 induction was observed between the non-deuterated and deuterated analogues (see Table 2). Over the 4-day period none of the compounds afforded significant cytochrome P-450 induction above that of control animals. A specific P-450 isozyme induction cannot be unequivocally ruled out on the basis of this limited study.

In conclusion, these isotope studies further substantiate that DDA, the major urinary metabolite of DDT, is formed via hydroxylation of the 1-carbon side chain of DDD in a process analogous to the metabolic disposition of chloroform.

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