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2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN: POTENT ANTICARCINOGENIC ACTIVITY IN CD-1 MICE[1]

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SUMMARY

Topical pretreatment with non-toxic doses of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin, a contaminant formed in the commercial synthesis of the herbicide 2, 4, 5-trichlorophenoxyacetic acid, strongly inhibited the initiation of skin tumors by 7, 12-dimethylbenz(a)anthracene and benzo(a)pyrene in female CD-1 mice. 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin also produced marked induction of epidermal monooxygenase enzymes functional in the conversion of 7, 12-dimethylbenz(a)anthracene to a variety of hydroxylated products. The time course of anticarcinogenic effects resulting from pretreatment with the dioxin correlated with the magnitude of induction as well as with a significant reduction in the quantity of 7, 12-dimethylbenz(a)anthracene metabolites covalently bound in vivo to epidermal DNA and RNA but not protein.

INTRODUCTION

Induction of microsomal enzymes in several tissues has been postulated as a mechanism for the anticarcinogenic effects of a wide variety of compounds. These include: PAH[3] (1-4), flavones (5-7), coumarins (8), phenothiazines (9), and pheno-

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[3]Abbreviations used are: PAH, polycyclic aromatic hydrocarbons; AHH, aryl hydrocarbon hydroxylase; HPLC, high pressure liquid chromatography; TCDD, 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin; TPA, 12-0-tetradecanoylphorbol-13-acetate; DDT, 1, 1, 1-trichloro-2, 2-bis-(p-chlorophenyl)ethane; 7-OHM-12-MBA, 7-hydroxymethyl-12-methylbenz(a)anthracene; 12-OHM-7-MBA, 12-hydroxymethyl-7-methylbenz(a)anthracene; 7, 12-diOHMBA, 7, 12-dihydroxymethylbenz(a)anthracene; 2-, 3-, 4- and 5-OH-DMBA, DMBA phenols, DMBA-cis-5, 6-diol, cis-5, 6-dihydro-5, 6-dihydroxy-7, 12-dimethylbenz(a)anthracene; DMBA-trans-8, 9-diol, trans-8, 9-dihydro-8, 9-dihydroxy-7, 12-dimethylbenz(a)anthracene; 7-OHM-12-MBA-trans-5, 6-diol, trans-5, 6-dihydro-5, 6-dihydroxy-7-hydroxymethyl-12-methylbenz(a)anthracene.

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barbital (10, 11). Induction of microsomal enzyme systems also has been implicated in the anticarcinogenic activity of DDT (12) and various polychlorinated biphenyl mixtures (13, 14). However, investigations such as these have not demonstrated conclusively that the inhibitory actions are a result of induction within the target tissue for a particular carcinogen or that the effect is on tumor-initiation per se.

TCDD is an extremely potent inducer of hepatic microsomal monooxygenase activity with properties similar to 3-methylcholanthrene (15). A remarkable feature of enzyme induction with TCDD is the duration of elevated enzyme activity. AHH (E.C. 1.14.14.2), a microsomal enzyme system responsible for converting PAH carcinogens to active as well as inactive metabolites, remains elevated for up to 35 days following a single intraperitoneal injection (15).

TCDD recently was shown to possess little or no tumor-initiating (16) or tumor-promoting properties (17) in mouse skin using the two-stage, initiation-promotion system of carcinogenesis. This finding suggested the use of TCDD as a tool for studying the effects of enzyme induction on tumor-initiation by PAH. A particular advantage of using the two-stage system of mouse skin carcinogenesis to study this phenomenon is that systemic metabolism of the carcinogen is not likely to affect the overall response since only very small doses of the carcinogen are applied locally. This report demonstrates that TCDD, at nontoxic doses, possesses remarkable inhibitory actions on skin tumor-initiation by PAH in CD-1 mice. This potent anticarcinogenic effect may be related to the ability of TCDD to induce epidermal enzyme pathways responsible for detoxifying PAH carcinogens in the skin.

MATERIALS AND METHODS

Chemicals. DMBA and BaP were obtained from the Sigma Chemical Co., St. Louis, MO. [^{3}H]DMBA (10.4 Ci/mmole) was purchased from Amersham/Searle, Arlington Heights, IL. TPA was supplied by Dr. Peter Borchert, University of Minnesota, Minneapolis, MN. TCDD was a gift from the Dow Chemical Co., Midland, MI. (98.6% pure by glc, Lot. # 851-144-2). Chemical derivatives of DMBA were kindly supplied by Drs. M.S. Newman, Ohio State University (Columbus, OH), H. V. Gelboin, National Cancer Institute (Bethesda, MD.), P. H. Jellinck, Queen's University (Kingston, Ontario) and P. L. Sims, Chester Beatty, Research Institute (London, England). These included 7-OHM-12-MBA, 12-OHM-7-MBA, 7,12-diOHMBA, 2-, 3-, 4- and 5-OH-DMBA, DMBA-cis-5,6-diol, DMBA-trans-8,9-diol and 7-OHM-12-MBA-trans-5,6-diol.

Tumor Experiments. Female outbred Charles River CD-1 mice were purchased from Charles River Mouse Farms, North Wilmington, MA. Mice 7-9 weeks old were carefully shaved with surgical clippers 2 days prior to initial treatment and only those mice in the resting phase of the hair cycle were used in the tumor experiments. Each experimental group contained 30 preshaved mice and all chemicals were applied topically to the shaved area of the back in 0.2 ml acetone. TCDD was applied at various time inter-

vals prior to initiation with DMBA or BaP. One week after initiation with the hydrocarbons tumors were promoted with topical applications of 10 μg TPA given twice weekly.

Metabolism of DMBA by Mouse Epidermal Homogenates. The metabolite profile of DMBA was analyzed using epidermal homogenates (obtained from control or TCDD-pretreated mice) as the enzyme source. Preparation of epidermal homogenates, assay conditions and analysis of metabolites using HPLC were performed as previously described (18).

Effects of TCDD-Pretreatment on Covalent Binding of ^{3}H DMBA to Epidermal Macromolecules. TCDD was applied topically to the shaved backs of CD-1 mice (1 ug/mouse) 3 days prior to application of 2.56 μg of [^{3}H]DMBA (10 μCi). Control animals were pretreated with 0.2 ml acetone. After topical application of [^{3}H]DMBA, mice were sacrificed 3 and 24 hours later. The epidermal material was removed by the heat treatment method (19) and DNA, RNA and protein were extracted using the method described by Huberman and Sachs (20).

RESULTS AND DISCUSSION

Table 1 illustrates the effects of topical pretreatment with TCDD on the initiation of skin tumors by DMBA. As presented in the table, the effect of TCDD was dependent on both the time of pretreatment as well as the dose. When applied 5 min prior to initiation with DMBA, a 2 μg topical dose of TCDD produced very little effect on the tumor response. However, with pretreatment times of 1, 3, 5 and 10 days, a 1 μg topical dose of TCDD resulted in 86, 91, 94 and 78% inhibition, respectively, in the number of papillomas formed per mouse. Maximum inhibition of tumor formation occurred when TCDD was applied 3 to 5 days prior to initiation with DMBA although significant inhibition was evident with the 10 day pretreatment. The time course of inhibition with TCDD corresponded with the time course of induction of monooxygenase activity in the skin (21), suggesting that the inhibitory effect might be related to enzyme induction.

To further analyze the inhibitory effect of TCDD, a dose response study was designed. These results are also depicted in Table 1. Three doses of TCDD were chosen; 0.01 μg, 0.10 μg and 2 μg/mouse and each dose was applied 3 days prior to initiation with DMBA. At doses of 0.01 μg, TCDD inhibited papilloma formation by approximately 80%, whereas a 0.1 μg dose of TCDD produced an approximately 92% inhibition. The 2 μg dose of TCDD was slightly more effective than the 0.1 μg dose. Except for the tumor experiments in which TCDD was applied at 2 μg doses, no toxicity was observed. Animals receiving 1 μg or less appeared normal in terms of weight gain and morphologic and histologic characteristics of the skin.

The effects of TCDD on tumor-initiation with BaP also were investigated to determine the generality of this inhibitory response. These results are presented in Table 2 and indicate a similar inhibitory effect of TCDD on skin tumor-initiation by BaP. However, the magnitude of the effect was slightly less than that observed with DMBA as the

TABLE I

INHIBITION BY TCDD OF DMBA SKIN TUMOR-INITIATION: DEPENDENCE ON TIME OF PRETREATMENT AND DOSE[a]

Pretreatment (ug/mouse)	Weeks of Promotion	Pretreatment Time	Papillomas Per mouse[b]	Percent of Control[c]
Acetone	20	5 min	3.80	100
TCDD (2)	20	5 min	3.70	97
TCDD (1)	20	1 day	0.53	14
TCDD (1)	20	3 days	0.34	9
TCDD (1)	20	5 days	0.23	6
TCDD (1)	20	10 days	0.85	22
Acetone	24	3 days	3.83	100
TCDD (0.01	24	3 days	0.65	17
TCDD (0.10)	24	3 days	0.31	8
TCDD (2)	24	3 days	0.14	4

[a] 30 mice were used per experimental group. Pretreated mice were initiated with 2.56 ug of DMBA and promoted twice weekly, for either 20 or 24 weeks, with 10 ug of TPA.

[b] Average number of papillomas per mouse after 20 or 24 weeks of promotion.

[c] The average number of papillomas per mouse expressed as a percentage of the DMBA-initiated, TPA-promoted groups for each period of promotion.

TABLE II

EFFECTS OF TCDD ON SKIN TUMOR-INITIATION BY BaP[a]

Pretreatment (ug/mouse)	Pretreatment Time	Papillomas Per Mouse[b]	Percent of Control[c]
Acetone	5 min	2.30	100
TCDD (1)	1 day	1.90	83
TCDD (1)	3 days	1.00	43
TCDD (1)	5 days	0.80	35

[a] 30 mice were used per experimental group. Pretreated mice were initiated with 100 nmoles of BaP and promoted with twice weekly applications of 10 ug of TPA. Promotion was continued for 20 weeks.

[b] Average number of papillomas per mouse after 20 weeks of promotion.

[c] Average number of papillomas per mouse expressed as a percentage of the BaP-initiated, TPA-promoted group.

initiating agent. The same dependence on the time of pretreatment was noted. A 1 μg dose of TCDD, given 5 days prior to initiation with BaP, produced an approximately 65% inhibition of papilloma formation.

Application of TCDD to the skins of genetically "inducible" as well as genetically "non-inducible" mouse strains results in marked increases in AHH activity in this tissue (22). A single topical application of TCDD (0.3 μg) to Swiss Webster CD-1 mice, produces an approximately 30-fold increase in AHH activity of the skin 3 days later (23). We have analyzed the metabolite profile of DMBA using epidermal homogenates obtained from TCDD-pretreated mice. TCDD was applied topically (1 μg per mouse) 3 days prior to sacrifice of the mice. Control mice received 0.2 ml of acetone. Epidermal homogenates from the TCDD-pretreated mice exhibited greatly increased rates of formation of hydroxylated products from DMBA (Figure 1). A comparison of profile A in Figure 1 (from control mice) with profile C (TCDD-pretreated mice) demonstrates greater proportionate increases in polyhydroxylated metabolites (e.g., diols) formed in tissues from TCDD-pretreated animals. Furthermore, several metabolite peaks (fractions 7, 10, 25 and 30) appear in profile C that are not detectably present in the profile from control tissues. Profile B of Figure 1 is added for comparison and was produced by pretreating mice with Aroclor 1254 (100 μg) 18 hours prior to sacrifice. Therefore, under conditions similar to those present in the tumor experiments with DMBA, both qualitative and quantitative differences exist with respect to metabolism of DMBA in this tissue.

To understand further the inhibitory effects of pretreatment with Aroclor 1254 and TCDD on skin tumor-initiation by DMBA, the effects of TCDD on covalent binding of ^{3}H-DMBA to mouse epidermal DNA, RNA and protein were investigated. These experiments are summarized in Table 3. TCDD was applied topically to CD-1 mice (1 μg per mouse) 3 days prior to application of ^{3}H-DMBA. After topical application of ^{3}H-DMBA, mice were sacrificed 3 and 24 hours later. Under these conditions (which mimicked the tumor experiments described above), TCDD-pretreatment was found to significantly reduce the covalent binding of DMBA to epidermal DNA (60-70%) and RNA (45-55%). Interestingly, TCDD pretreatment had no effect on the amount of hydrocarbon covalently bound to epidermal proteins.

The unusually potent inhibitory action of TCDD on skin tumor-initiation by DMBA and BaP stands in contrast to several reports by Kouri et al. (21, 24). TCDD was found to enhance the formation of MC-induced sarcomas at the site of subcutaneous injection when the two compounds were administered concurrently to "non-responsive" D-2 mice. This effect was attributed to the ability of TCDD to enhance the metabolism of MC in

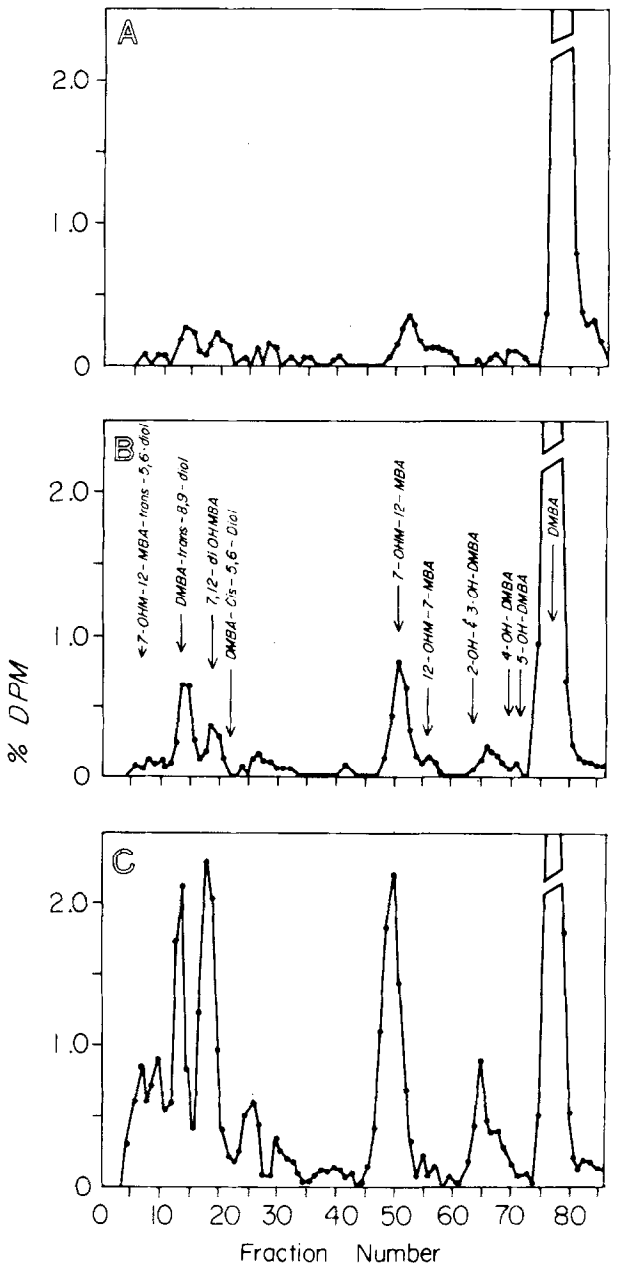

Figure 1. High-pressure liquid chromatographic profiles of ^{14}C-DMBA metabolites generated in vitro using epidermal homogenates from variously pretreated female CD-1 mice. Profile A; mice were pretreated topically with 0.2 ml acetone 18 hours prior to sacrifice. Due to extremely low activity in homogenates from uninduced mice, DPM values for each fraction collected before fraction 75 (in Profile A) were multiplied by a factor of 3 to permit display of the data as % DPM. Profile B; mice were pretreated topically with 100 μg of Aroclor 1254 in 0.2 ml acetone 18 hours prior to sacrifice.

TABLE III

EFFECT OF PRETREATMENT WITH TCDD ON COVALENT BINDING OF ^{3}H-DMBA TO MOUSE EPIDERMAL DNA, RNA AND PROTEIN

Mice were treated topically with 2.56 ug of ^{3}H-DMBA (10 uCi) and sacrificed 3 and 24 hours later.

Treatment	Time (hr)[a]	Hydrocarbon bound to macromolecule (pmoles/mg x 10^{2})[b] DNA	RNA	Protein
DMBA	3	4.79	2.88	69.5
DMBA	24	12.63	7.16	38.8
DMBA + TCDD[c]	3	1.78 (37)	1.56 (54)	63.8
DMBA + TCDD[c]	24	3.52 (28)	3.16 (44)	39.4

[a]Time of sacrifice after application of ^{3}H-DMBA; 40 mice were used for each experimental group.

[b]Numbers in parentheses are percentages of values found in the same time group not receiving TCDD pretreatment.

[c]Mice in this group received 1 ug of TCDD, applied topically, 3 days prior to application of ^{3}H-DMBA.

this system. However, they found that when given simultaneously with the initiator to C57BL/6N mice, TCDD did not exhibit an effect and in some cases actually inhibited tumorigenesis when the mice were pretreated. These results would tend to agree with those obtained in our experiments. Further work will be required to clarify the discrepancies between the results obtained with these two test systems (initiation-promotion on mouse skin vs. subcutaneous sarcoma formation) as well as metabolic differences between various mouse strains and tissues.

The anticarcinogenic effect of TCDD appears to correlate with the ability of this compound to induce monooxygenase enzymes of the skin. Furthermore, the quantity of ^{3}H-DMBA bound to DNA and RNA (but not protein) in the presence and absence of TCDD-pretreatment correlated well with the tumor response under similar conditions. When taken together, these experiments suggest that pretreatment with TCDD gives rise to an increased rate of inactivation of the DMBA molecule relative to the rate of activation in mouse skin.

Profile C; mice were pretreated topically with 1 µg of TCDD 3 days prior to sacrifice. The retention times of various synthetic standard compounds (either known or suspected metabolites of DMBA) are depicted as arrows above peak fractions of Profile B in which they elute in this system. Further details of the methodology can be found in reference 18.

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