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Dibenzoylmethane Modulates Aryl Hydrocarbon Receptor Function and Expression of Cytochromes P450 1A1, 1A2, and 1B1

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ABSTRACT

The phytochemical dibenzoylmethane (DBM) has been shown to prevent polycyclic aromatic hydrocarbon (PAH)-induced tumorigenesis in rodents. However, the biochemical basis of this activity is unclear. We have therefore investigated the effects of DBM on the activity and expression of carcinogen-activating enzymes, the cytochromes P450 (CYP) 1A1, 1A2, and 1B1. Oral administration of DBM to female Sprague Dawley rats inhibited the increase in hepatic enzyme activity and mRNA levels of CYP1A1, 1A2, and 1B1 caused by the PAH 7,12-dimethylbenz[*a*]anthracene (DMBA). However, DBM administration alone caused an increase in both activity and expression in the liver, albeit to levels much lower than that induced by DMBA. To characterize the molecular mechanisms involved in this dual action of DBM, we examined the effects of DBM *in vitro*. In HepG2 human hepatoma cells, DBM inhibited DMBA- and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-induced enzyme activity and CYP1A1, 1A2, and 1B1 mRNA levels, whereas DBM itself induced activity and mRNA expression. Modulation of *CYP1A1* expression by DBM occurred at the transcriptional level, as transient transfection assays demonstrated. Because the transcription of *CYP1A1* is regulated by the aryl hydrocarbon receptor (AhR), we investigated the effect of DBM on AhR activation. DBM inhibited TCCD-induced DNA-binding of the AhR to the xenobiotic-responsive element (XRE) of *CYP1A1* as measured by electrophoretic mobility shift assay. These data suggest that the chemopreventive activity of DBM results from its ability to affect Phase 1 enzyme expression by modulation of AhR function.

INTRODUCTION

DMBA[2] (Fig. 1) is a member of a class of compounds referred to as PAHs, which require bioactivation to their ultimate carcinogenic forms. The activation pathway is mediated by a cytosolic transcription factor, the AhR. Consequent to ligand [PAHs or halogenated derivatives, *e.g.*, TCDD (Fig. 1)] -binding, the AhR:ligand complex translocates to the nucleus where it binds another protein, the AhR nuclear translocator. Upon association with the AhR nuclear translocator, the AhR is converted to a high-affinity DNA-binding form (1). This complex subsequently binds a sequence, the XRE, found within the enhancer region of a variety of genes. The best-characterized AhR-mediated biochemical response is the induction of *CYP1A1*, which encodes the enzyme CYP1A1. CYP1A1 converts many PAHs into genotoxic metabolites, which can covalently bind to intracellular nucleophiles, including DNA, and initiate the cancer process. Decreased metabolic activation of carcinogens via direct inhibition of CYP1A1 activity or through inhibition of the AhR activation pathway is believed to be an important mechanism of cancer prevention. In addition to CYP1A1, two other carcinogen-activating CYPs, or so-called Phase 1 enzymes, are also regulated by the AhR. *CYP1A2*, primarily expressed in the liver, metabolizes a number of important drugs (2) as well as many procarinogens, including PAHs, nitrosamines, and arylacetamides (3). *CYP1B1* is widely expressed, and, similar to *CYP1A1* and *CYP1A2*, is inducible by TCDD and PAHs in both rodents and humans (4, 5). It has been reported that CYP1B1 activates both PAHs and aryl amines (4). Therefore, inhibition of CYP1A2 and CYP1B1 may also play a role in cancer prevention.

DBM (Fig. 1) is a phenolic constituent of licorice that has antimutagenic activity. It has been demonstrated that DBM inhibits the mutagenicity of 2-naphtholhydroxamic acid, benzo(*a*)pyrene, and aflatoxin B_1 in *Salmonella typhimurium* (6). Singletary *et al.* (7) have shown that DBM has potent chemopreventive activity against DMBA-induced carcinogenesis in rats. This effect was attributed to, in part, the ability of DBM to inhibit DMBA-DNA adduct formation. However, the molecular and biochemical mechanisms of the chemopreventive activity of DBM have not been fully elucidated. Therefore, in the present study, we investigated the effects of DBM on various aspects of the carcinogen activation pathway mediated by the AhR.

MATERIALS AND METHODS

Materials. HepG2 cells were from American Type Culture Collection (Rockville, MD). RPMI 1640, TRIzol, and Lipofectamine were from Life Technologies, Inc. (Grand Island, NY). Actinomycin D, DMBA, polydeoxyinosinic-deoxycytidylic acid, ERF, resorufin, and DBM were from Sigma Chemical Co. (St. Louis, MO). [^{32}P]dATP was from NEN Life Science Products (Boston, MA). TCDD was from the Midwest Research Institute (Kansas City, MO). The Omniscript kit was from Qiagen (Valencia, CA). Tris-borate gels, Tris-borate running buffer, and high-density sample buffer were from Novex (San Diego, CA). The CAT ELISA kit was from Roche Molecular Biochemicals (Indianapolis, IN).

Animal Studies. Female Sprague Dawley rats (Charles Rivers Breeding Laboratories, Wilmingon, MA) were received at 4 weeks of age and maintained on a 12 h light/dark cycle. Animals were provided with an AIN76A semipurified powder diet and water *ad libitum*. Beginning at 5 weeks of age, their diet was supplemented every other day with vehicle (olive oil, $n = 8$), or DBM at 100 ($n = 8$) or 200 mg/kg body weight ($n = 8$), delivered by gavage. On the eighth supplementation, animals were treated with vehicle control (DMSO) or DMBA (5 mg/kg) in addition to DBM. All test compounds were dissolved on the day of use in DMSO. The animals were killed 24 h later, and liver tissue was excised, quick frozen, and stored at −80°C until analyzed.

Cell Culture. HepG2 human hepatoma cells were grown with RPMI 1640 supplemented with 2 mM glutamine and 10% fetal bovine serum in a 5% CO_2 humidified incubator at 37°C. For all treatment compounds, DMSO was used as the vehicle and did not exceed 0.1%.

Assay of Phase I Enzyme Activity in Rat Liver and HepG2 Cells. Phase 1 enzyme activity from rat liver was determined by EROD activity assay in the following manner: 5 μg of microsomes were brought up to 100 μl with PBS (pH 7.2). ERF (400 nM) was added, and the reaction was initiated by the addition of 0.5 M NADPH. The reaction mixture was transferred to a 96-well plate, and enzyme activity was determined in a CytoFluor multiwell fluorescence plate reader, Series 4000 (PerSeptive Biosystems, Framingham, MA), with an excitation wavelength of 530 nm and emission at 590 nm. The reaction

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[2] The abbreviations used are: DMBA, 7,12-dimethylbenz[*a*]anthracene; PAH, polycyclic aromatic hydrocarbon; AhR, aryl hydrocarbon receptor; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; XRE, xenobiotic-responsive element; CYP1A1, cytochrome P4501A1; CYP1A2, cytochrome P4501A2; CYP1B1, cytochrome P4501B1; DBM, dibenzoylmethane; DMSO, dimethylsulfoxide; ERF, ethoxyresorufin; EROD, ethoxyresorufin-*O*-deethylase; G3PDH, glyceraldehyde-3-phosphate dehydrogenase; RT-PCR, reverse transcription-PCR; CAT, chloramphenicol acetyltransferase; EMSA, electrophoretic mobility shift assay.

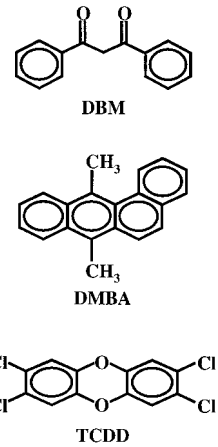

Fig. 1. Structures of DBM, DMBA, and TCDD.

was allowed to run for 30 min. A standard curve was constructed using resorufin. The effect of DBM on Phase 1 carcinogen activating capacity was also evaluated in whole HepG2 cells by measurement of EROD activity as described by Kennedy and Jones (8).

RT-PCR. Total RNA was isolated from a small piece of liver that had been subjected to Polytron homogenization, or from HepG2 cells, using TRIzol as directed. cDNA synthesis, semiquantitative RT-PCR for rat or human CYP1A1, 1A2, 1B1, and G3PDH, and analysis of results were performed as described previously (9). Primer sequences for human CYP1A1 and 1B1 were from Dohr *et al.* (10), and human CYP1A2 from Chung and Bresnick (11). Sequences for rat CYP1A1, 1A2, and 1B1 were from Walker *et al.* (12), and sequences for rat and human G3PDH were from Clontech (Palo Alto, CA). For the PCR, the optimum cycle number that fell within the exponential range of response for rat CYP1A1 (22 cycles), rat CYP1A2 (18 cycles), rat CYP1B1 (28 cycles), rat G3PDH (18 cycles), human CYP1A1 (22 cycles), human CYP1A2 (28 cycles), hCYP1B1 (29 cycles), or hG3PDH (17 cycles) was used.

EMSA. HepG2 cells were incubated with DMSO (0.1%), 10 nM TCDD, 10 nM TCDD + 1 μM DBM, or 20 μM DBM (alone) for 2 h at 37°C. Nuclear protein was isolated, and EMSA was performed by the method of Denison *et al.* (13).

Transcription Assay. HepG2 cells were seeded onto 24-well plates at 100,000 cells/well. After 24 h, the cells were transiently transfected with a CAT reporter vector (0.25 μg/well) containing the full-length *CYP1A1* promoter (pMC6.3) and a β-galactosidase vector (0.02 μg/well) with the use of Lipofectamine. Transfected cells were treated with various concentrations of DBM in the presence of either 375 pM TCDD or 2 μM DMBA, or with DBM alone. *CYP1A1* promoter-controlled CAT transcription in HepG2 cells was determined as described previously (9).

Ligand-binding Assay. Cytosolic fractions of HepG2 cells were prepared as published previously (14), and AhR ligand-binding was measured according to the methods of Raha *et al.* (15). Cytosolic protein (1.5 mg/500 μl) was incubated with 20 nM [^{3}H]-TCDD simultaneously with either DMSO (5 μl/500 μl), 50 μM DBM, or 10 μM TCDD (unlabeled) for 2 h at 4°C. Samples were centrifuged in linear gradients of 5–30% sucrose (v/v) in 12-ml Beckman Quick-Seal rotor tubes at 65,000 rpm for 2 h at 4°C in a Beckman VTI-65–1 rotor. Fractions (~500 μl each) of the gradients were collected, and radioactivity was determined by scintillation counting.

Statistical Analysis. Statistical analyses were performed with STATVIEW Statistical Analysis Software (SAS Institute, San Francisco, CA). Differences between group mean values were determined by a one-factor ANOVA, followed by Fisher PSLD post hoc analysis for pairwise comparison of means.

RESULTS

Effect of DBM on Phase I Enzyme Activity in Rat Liver. Carcinogen activating capacity was measured as EROD activity in microsomes isolated from livers of female Sprague Dawley rats. DMBA caused a 35-fold increase in activity in rat liver microsomes compared with controls (Fig. 2*A*). Administration of DBM before DMBA administration suppressed this inductive effect in a concentration-dependent manner (Fig. 2*A*). In liver microsomes of animals treated with DBM alone, there was a concentration-dependent increase in EROD activity compared with controls (Fig. 2*B*).

The direct effect of DBM on EROD activity was determined on microsomal fractions from livers of DMBA-treated rats. DBM treatment resulted in a concentration-dependent decrease in EROD activity, with an IC_{50} of approximately 1.1 μM (Fig. 3*A*). We examined further the effect of DBM on microsomal EROD activity in the presence of different substrate concentrations. Results are illustrated in the form of a Hanes-Woolf plot (Fig. 3*B*) and reveal an increase in K_m with increasing DBM concentrations, whereas the $\mathbf{V}_{max}$ is unaffected, indicating that DBM is a competitive inhibitor. The inhibition constant (K_i) for DBM was calculated to be ~0.27 μM by linear regression analysis.

Effect of DBM on CYP1A1, 1A2, and 1B1 mRNA Expression in Rat Liver. DMBA induced hepatic CYP1A1, 1A2, and 1B1 mRNA expression by 17.2-, 11.5-, and 5.5-fold above basal levels, respectively (Fig. 4*A*). The induction of mRNA expression of each of these CYPs was inhibited by DBM in a concentration-dependent manner (Fig. 4*A*). Administration of DBM alone caused a concentration-dependent increase in CYP1A1, 1A2, and 1B1 mRNA expression (Fig. 4*B*).

Effect of DBM on Phase I Enzyme Activity in HepG2 Cells. In HepG2 cells treated with the potent AhR ligand TCDD, the specific EROD activity was 468.66 ± 21.64 fmol/min/well. The addition of DBM led to a concentration-dependent decrease in EROD activity (Fig. 5*A*), with an IC_{50} of 0.5 μM. DBM also inhibited EROD activity in cells induced with DMBA, IC_{50}, ~0.07 μM (Fig. 5*B*). By itself, at concentrations ≥1.0 μM, DBM induced EROD activity (Fig. 5*C*). DBM had no effect on cell growth at any of the concentrations tested, as determined by sulforhodamine B assay (not shown).

Effect of DBM on CYP1A1, 1A2, and 1B1 mRNA Levels in HepG2 Cells. In HepG2 cells, TCDD caused a 7.9-, 2.6-, and 3.3-fold increase in CYP1A1, 1A2, and 1B1 mRNA expression, respectively, compared with controls. These increases were abated in a concentration-dependent fashion with DBM cotreatment (Fig. 6*A*).

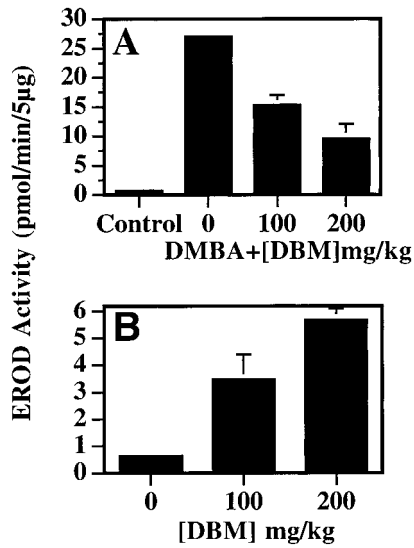

Fig. 2. Effect of DBM on Phase I enzyme activity in rat liver. Rats were given DBM and then a single dose of DMBA (*A*), or they were given DBM alone (*B*), as described in "Materials and Methods." Microsomes were isolated from livers, and the Phase I enzyme activity was determined by EROD assay as described in "Materials and Methods." $n = 4$; *bars,* SE.

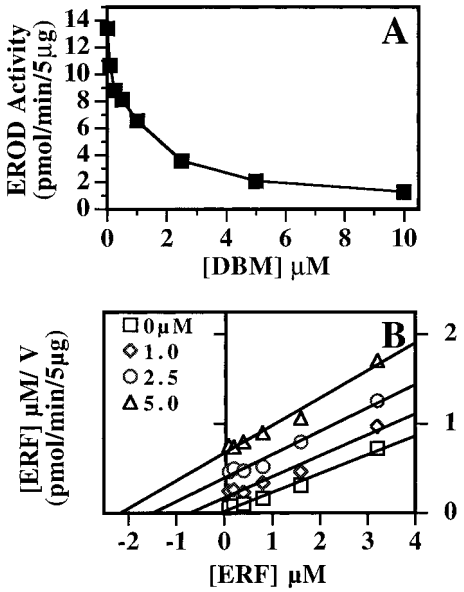

Fig. 3. Direct effect of DBM on microsomal EROD activity. Microsomes isolated from the livers of DBMA-treated rats were used to determine the direct effect of DBM on EROD activity. Activity was measured in the presence of different concentrations of DBM (*A*), $n = 4$; *bars*, SE (SE < symbol size); and in the presence of different concentrations of both DBM and ERF (*B*) expressed as a Hanes-Woolf plot. $n = 4$.

The inductive effect of TCDD was significantly decreased in cells cotreated with 0.25 μM DBM, and completely abolished in cells treated with 1 μM DBM. DBM was equally effective in inhibiting DMBA-induced increases in CYP1A1, 1A2, and 1B1 mRNA levels (not shown).

When DBM was given alone, lower concentrations (0.5 and 1.0 μM) resulted in a reduction of CYP1A1, 1A2, and 1B1 mRNA expression in HepG2 cells (Fig. 6*B*). In contrast, higher concentrations of DBM (5, 10, and 20 μM) caused a concentration-dependent increase in CYP1A1 and CYP1B1 mRNA expression (Fig. 6*B*). The effect on CYP1A2 was similar, although a significant increase in mRNA expression was only observed at the 10- and 20-μM concentrations of DBM.

Effect of Actinomycin D on DBM-induced CYP1A1 mRNA Expression. Actinomycin D chase experiments were performed using HepG2 cells to determine whether DBM induces CYP1A1 mRNA by a transcriptional mechanism. Whereas the effect of DBM treatment alone resulted in a 4-fold increase in CYP1A1 mRNA expression, pretreatment with actinomycin D blocked this effect (Fig. 7).

Effect of DBM on CYP1A1 Transcription. HepG2 cells were transfected with the *CYP1A1* promoter element fused to a CAT reporter gene. TCDD induced CAT activity by 3.4-fold above control levels. The addition of DBM caused a concentration-dependent diminution of CAT activity to levels approximately equal to that of control at 1.0 μM (Fig. 8*A*). The induction of CAT activity by DMBA of 1.67-fold above control levels was less than that by TCDD, and at all concentrations, DBM suppressed this induction of CAT activity (not shown). Treatment with DBM alone led to a 2.34-fold increase in CAT activity compared with controls (Fig. 8*B*).

Effect of DBM on the Activation of AhR in HepG2 Cells. We evaluated the effect of DBM on activation of the AhR in HepG2 cells by EMSA. TCDD (10 nM) caused an increase in AhR activation, which was suppressed by 1 μM DBM, whereas 20 μM DBM caused an increase in activation of the AhR (Fig. 9). The specificity of this bandshift was confirmed by the inhibition of the bandshift in nuclear extracts treated with a 15-fold excess of unlabeled XRE (not shown).

Effect of DBM on the Binding of Ligand to the AhR. Competitive binding assays were performed using cytosolic fractions from HepG2 cells. Treatment with 20 nM [^{3}H]-TCDD resulted in a 9S peak that was competitively inhibited with excess unlabeled TCDD or with 2500-fold molar excess of DBM (Fig. 10).

DISCUSSION

DBM is a natural compound that has recently emerged as a potential chemopreventive agent. It has been reported that dietary DBM is a potent inhibitor of chemically induced mammary carcinogenesis and PAH-DNA adduct formation in rodents (7, 16). An increase in the Phase II enzymes glutathione-S-transferase and quinone reductase has also been evidenced in livers of animals given DBM-supplemented diets (7). However, in human mammary epithelial cells (MCF-10F), there was no significant induction of glutathione-S-transferase by DBM and only a slight increase in quinone reductase protein levels at the highest concentration of DBM tested (2 μM) despite potent inhibition of benzo(*a*)pyrene-DNA adduct formation by DBM (17). Hence, the chemopreventive activity of DBM must involve additional

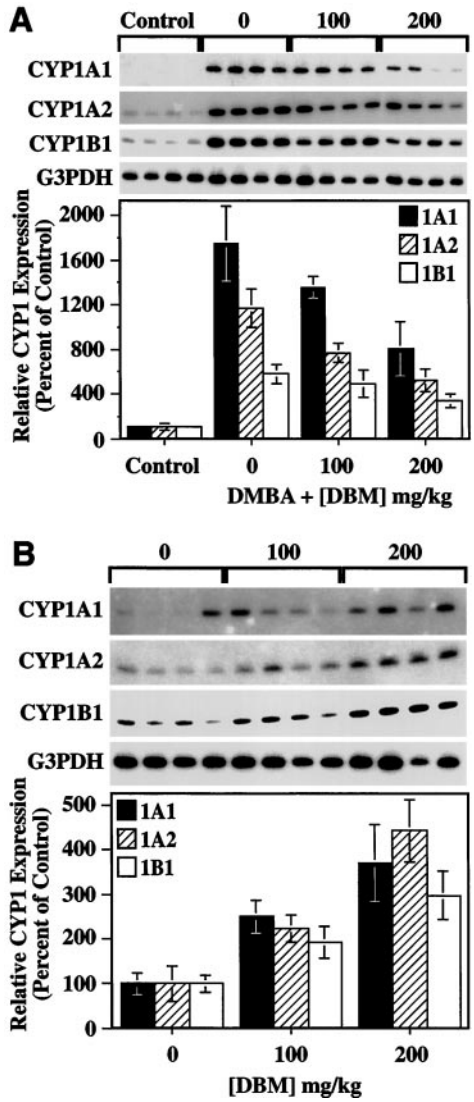

Fig. 4. Effect of DBM on CYP1A1, 1A2, and 1B1 mRNA levels. Rats were given DBM and then a single dose of DMBA (*A*), or they were given DBM alone (*B*) as described in "Materials and Methods." CYP1A1, CYP1A2, CYP1B1, and G3PDH mRNA were determined by RT-PCR. CYP mRNA was normalized to G3PDH mRNA. $n = 4$; *bars*, SE.

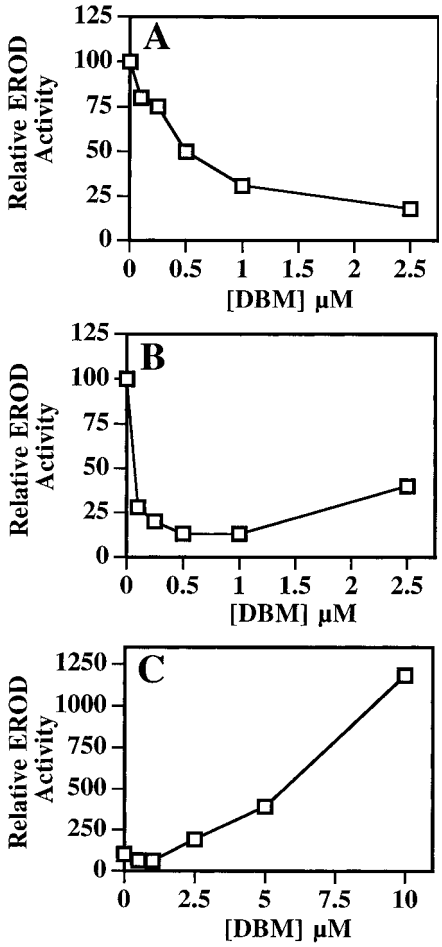

Fig. 5. Effect of DBM on Phase I enzyme activity in HepG2 cells. Phase I enzyme activity was measured by EROD assay in intact cells. Cells were treated with 100 pM TCDD (*A*) or with 1 μM DMBA (*B*) with or without the indicated concentrations of DBM, or cells were treated with DBM alone (*C*). $n = 4$; *bars*, SE (SE < symbol size).

mechanisms. We have, therefore, evaluated the role of DBM in the regulation of the carcinogen activation pathway mediated by the AhR.

DMBA is a potent inducer of carcinogenesis in rodents. In our animal study, DMBA caused a potent induction of EROD activity in rat liver that was significantly inhibited by DBM (Fig. 2*A*). The inhibitory effect on EROD activity by DBM could be accounted for by two modes of action. First, DBM directly inhibited Phase I enzyme activity in microsomes of DMBA-treated rats (Fig. 3*A*) in a competitive manner, as demonstrated by kinetic analysis (Fig. 3*B*). Second, the induction of CYP1A1, 1A2, and 1B1 mRNA expression by DMBA was also inhibited by DBM (Fig. 4*A*). Our results are the first demonstration, to our knowledge, of a modulation of CYP1B1 expression by a naturally occurring compound. This is particularly important in light of recent evidence concerning the activity of CYP1B1 toward several biochemical processes important to carcinogenesis. Although CYP1A isozymes are generally regarded as the predominant P450s involved in PAH activation, in recent years the importance of CYP1B1 in this respect has also been demonstrated (18, 19). Indeed, it has been suggested that CYP1B1 possesses a greater capacity than CYP1A1 to bioactivate a number of PAH procarcinogens (4). Furthermore, CYP1B1 is expressed in some tumor types, but not in the corresponding normal tissue (20), and thus may play a role in cellular transformation beyond PAH activation (21, 22). CYP1B1 has also been shown to convert estradiol to a carcinogenic form (23), indicating a possible role in mammary carcinogenesis (24). Thus, the present results may also be important in areas other than PAH activation.

In the livers of animals that were given DBM alone, we observed a modest increase in EROD activity (Fig. 2*B*). Livers from rats that were given DBM alone also had increased CYP1A1, 1A2, and 1B1 mRNA expression (Fig. 4*B*). Because CYP1A1, 1A2, and 1B1 expression are under the transcriptional regulation by the AhR, these results suggest that DBM acts via the AhR. We hypothesized that DBM might itself be a ligand of the AhR, and that in the presence of a more potent ligand, such as DMBA or TCDD, DBM functions as an inhibitor of the AhR.

To test this hypothesis, we investigated the effects of DBM on TCDD- and DMBA-induced EROD activity and CYP1A1, 1A2, and 1B1 mRNA expression in cultured liver cells (HepG2). The HepG2 cell-line is derived from human liver cancer cells and has been extensively used in studies of AhR-mediated carcinogen activation (25–28). TCDD is the most potent ligand known of the AhR and is the best-characterized inducer of CYP transcription. DBM inhibited TCDD-induced EROD activity (Fig. 5*A*) and CYP1A1, 1A2, and 1B1 mRNA expression (Fig. 6*A*). Additionally, DBM caused a marked suppression of DMBA-induced EROD activity (Fig. 5*B*) and CYP1A1

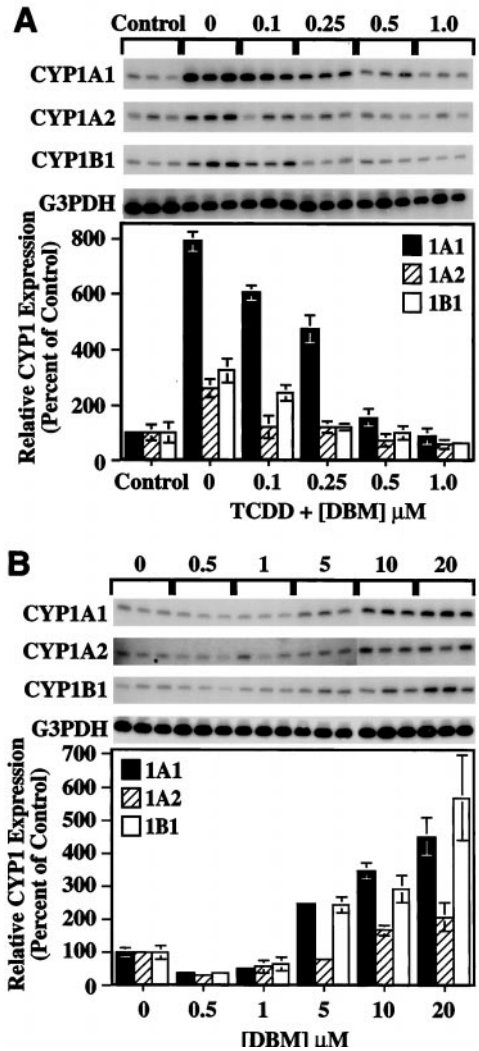

Fig. 6. Effect of DBM on CYP1A1, 1A2, and 1B1 mRNA expression in HepG2 cells. HepG2 cells were treated with 100 pM TCDD (*A*) in the presence of various concentrations of DBM, or cells were treated with DBM alone (*B*) for 8 h. CYP1A1, 1A2, 1B1, and G3PDH mRNA were determined by RT-PCR. CYP mRNA was normalized to G3PDH mRNA. $n = 3$; *bars*, SE.

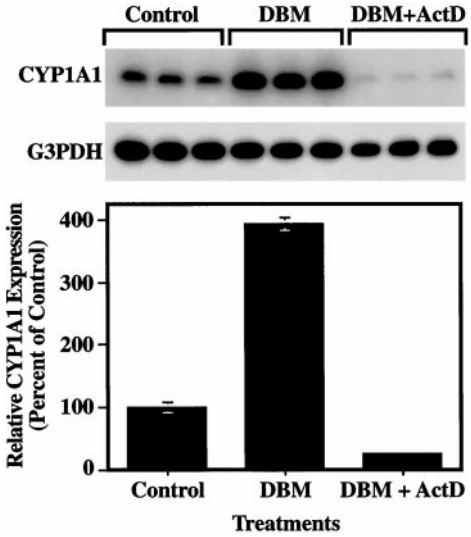

Fig. 7. Effect of actinomycin D on DBM-induced CYP1A1 mRNA expression. HepG2 cells were treated with actinomycin D (5 μg/ml) or with ethanol vehicle for 1 h and then treated for an additional 8 h with 20 μM DBM with or without actinomycin D. CYP1A1 and G3PDH mRNA were determined by RT-PCR. CYP1A1 mRNA was normalized to G3PDH mRNA. $n = 3$; *bars*, SE.

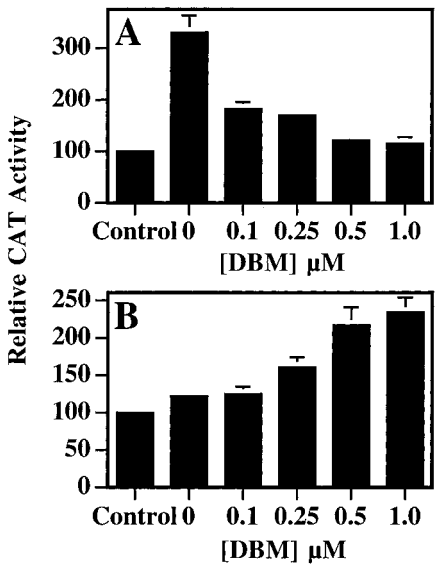

Fig. 8. Effect of DBM on CAT transcription mediated by the *CYP1A1* promoter. HepG2 cells were transiently transfected with a CAT reporter vector (0.25 μg/well) containing the full-length *CYP1A1* promoter and a β-galactosidase vector. Transfected cells were treated with 375 pM TCDD (*A*) in the presence of various concentrations of DBM, or cells were treated with DBM alone (*B*) for 6 h. CAT transcription was normalized to β-galactosidase transcription. $n = 4$; *bars*, SE.

mRNA expression (not shown) in HepG2 cells, similar to what was seen in the *in vivo* studies, indicating that DBM has equipotent inhibitory capacity against two types of PAHs. Administration of low concentrations of DBM alone caused a decrease in basal EROD activity (Fig. 5*C*) and CYP1A1, 1A2, and 1B1 mRNA expression (Fig. 6*B*), whereas higher concentrations of DBM resulted in increases for each of these measures (Fig. 6*B*).

We attribute the changes in mRNA expression to a direct effect on transcription. Support for this comes from 2 lines of evidence. (*a*) Although DBM (20 μM) caused an increase in CYP1A1 mRNA, DBM was unable to induce expression in the presence of the transcription inhibitor actinomycin D (Fig. 7); and (*b*) transient transfection assays demonstrated the inhibitory effects of DBM on TCDD-induced CYP1A1 transcription (Fig. 8*A*) and an inductive effect on transcription by DBM alone (Fig. 8*B*) that were similar to the mRNA expression data. DBM also inhibited DMBA-induced CYP1A1 transcription (not shown).

The contrary inhibitory and inductive capacities of DBM led us to examine the potential of DBM to affect AhR activation and to function as a ligand for the AhR. Using EMSA, we showed that 10 nM TCDD caused an increase in the amount of [^{32}P]-labeled XRE binding to the AhR, and this effect was attenuated by the addition of 1 μM DBM, whereas a high concentration of DBM (20 μM) activates the DNA-binding capacity of the AhR (Fig. 9). Furthermore, in the ligand-binding assay, we demonstrated that DBM (2500-fold molar excess) completely inhibited TCDD-binding to the AhR (Fig. 10). Our data provide strong support that DBM is a ligand of the AhR, although it has relatively weak affinity for the receptor compared with TCDD or DMBA. It is this property that likely enables DBM to compete with more potent inducing agents for the AhR.

It is interesting to note that, in HepG2 cells treated with DBM alone, there is a decrease in the basal levels of both EROD activity (Fig. 5*C*) and mRNA expression (Fig. 6*B*) in cells treated with <1 μM DBM. The basal expression of CYPs is believed to be regulated by the constitutive activation of the AhR by endogenous ligands, which are unidentified as yet (29). It is possible that DBM interferes with the binding of these ligands. Furthermore, a growing body of evidence indicates that CYP1A1 transcription may also be controlled through a ligand-independent mechanism involving a tyrosine kinase-mediated pathway (30, 31). Whether DBM participates in this signal transduction-mediated pathway is currently under investigation.

In summary, our studies suggest that DBM is a natural ligand of the

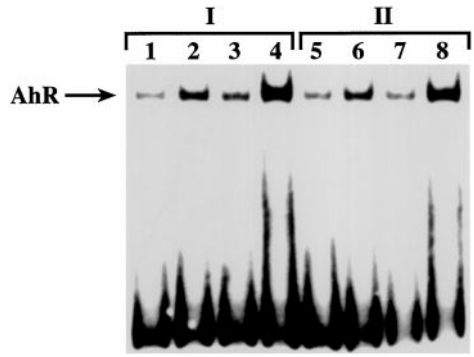

Fig. 9. Effect of DBM on AhR activation. HepG2 cells were incubated with DMSO (0.1%; *1* and *5*), 10 nM TCDD (*2* and *6*), 10 nM TCDD + 1 μM DBM (*3* and *7*), or 20 μM DBM (alone; *4* and *8*) for 2 h at 37°C. Nuclear protein was isolated, and the amount of activated AhR in10 μg of protein was measured by EMSA. Results are representative of two separate experiments.

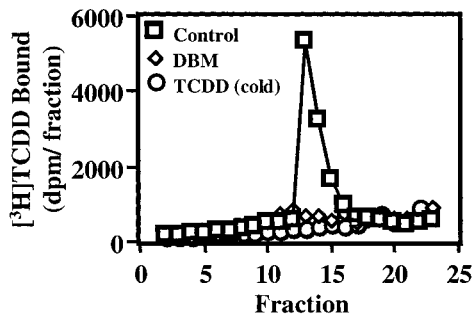

Fig. 10. Effect of DBM on the binding of [^{3}H]-TCDD to the AhR. Cytosolic fractions (1.5 mg/500 μl) of HepG2 cells were incubated with 20 nM [^{3}H]-TCDD in the presence of DMSO (5 μl/500 μl), 50 μM DBM, or 10 μM TCDD (*cold*) for 2 h at 4°C. The specific binding of [^{3}H]-TCDD was measured by sucrose density gradient centrifugation. Results are representative of three separate experiments.

AhR, and its interaction with the receptor serves to modify carcinogen-induced Phase 1 enzyme expression and activity by modulating AhR function. Therefore, the chemopreventive activity of DBM that has been demonstrated in previous studies may result from this effect.

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