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Relative importance of maternal and embryonic microsomal epoxide hydrolase in 7,12-dimethylbenz[*a*]anthracene-induced developmental toxicity

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Abstract

Microsomal epoxide hydrolase (mEH) catalyzes the hydrolysis of epoxide intermediates derived from drugs and environmental chemicals. The response of *in vivo* (embryo) and *in vitro* (embryo fibroblast) tests were analyzed using mEH-null and wild-type mice to determine the relative role of maternal and embryonic mEH in the developmental toxicity induced by 7,12-dimethylbenz[*a*]anthracene (DMBA). Embryos derived from DMBA-treated [50 mg/kg, daily from gestational day (GD) 11 to GD 15] dams were analyzed. Although weight ($P = 0.0009$) and crown-rump length ($P = 0.0003$) of wild-type fetuses on GD 18 were significantly lower than those of mEH-null fetuses, respectively, no significant difference was found between mEH-null and heterozygous fetuses of mEH-null dams. Cell viability was decreased to 50% in wild-type mouse embryo fibroblasts (MEFs) treated with 3 μM DMBA, but no significant decrease was found in mEH-null MEFs. DMBA-3,4-diol produced a significant decrease in cell viability and suppressed the proliferation of wild-type MEFs at a 10-fold lower concentration than did DMBA. Although mEH protein was expressed in liver microsomes from wild-type embryos (GD 15), DMBA-3,4-diol was not detected among the DMBA metabolites. However, it was detected in the serum of wild-type pregnant mice treated with DMBA, but not in that of mEH-null mice. These results suggest that maternal mEH plays a major role in DMBA-induced developmental toxicity, and embryonic mEH is less involved in the toxicity. © 2002 Elsevier Science Inc. All rights reserved.

Keywords: Developmental toxicity; 7,12-Dimethylbenz[*a*]anthracene; Microsomal epoxide hydrolase; Gene knockout mice; Embryo fibroblast; Embryo

1. Introduction

Numerous studies have shown the involvement of drug-metabolizing enzymes in chemical-induced developmental toxicity. Xenobiotic-metabolizing enzymes are expressed in embryos [1,2] during the organogenesis stage, a critical period that exhibits the highest degree of sensitivity to toxic chemicals. To understand the underlying mechanism of chemical-induced developmental toxicity, it is necessary to examine xenobiotic-metabolizing enzymes in maternal and fetal tissue. It is, however, difficult to distinguish between the contribution of embryonic xenobiotic-metabolizing enzymes and maternal enzymes using *in vivo* assays. Recently, several mouse lines lacking xenobiotic-metabolizing enzymes were established and used to determine the mechanism of chemical toxicity [3–5]. These mice can also be useful tools to determine the role of embryonic xenobiotic-metabolizing enzymes in embryo toxicity, since it is possible to distinguish between embryonic and maternal genotype.

mEH plays a central role in the metabolic transformation of many aliphatic epoxides and arene oxides derived from drugs and environmental chemicals [6,7]. mEH is expressed not only in liver, but also in several extrahepatic tissues including the placenta, uterus, and embryo [8,9]. mEH protein and activity were detected during the period

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Abbreviations: CYP, cytochrome P450; DMBA, 7,12-dimethylbenz[*a*]anthracene; GD, gestational day; MEF, mouse embryo fibroblast; mEH, microsomal epoxide hydrolase; and PAH, polyaromatic hydrocarbon.

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of organogenesis in humans and rats [10,11], suggesting the possibility that mEH is involved in the metabolism of teratogens at the target organ. The anticonvulsant drugs, phenytoin and carbamazepine, known teratogens, are thought to cause their harmful effects through arene oxide reactive intermediates [12–15]. Buehler *et al*. [16] reported a correlation between mEH activity and risk for congenital malformations induced by anticonvulsant drugs. On the other hand, PAHs in cigarette smoke, which give rise to embryo toxicity and carcinogenesis, are activated metabolically to a diol-epoxide by CYP and mEH. Thus, hydrolysis of the PAH epoxide carried out by mEH is considered a key step in metabolic activation [17–20]. One of the PAHs, DMBA, is used extensively as a model carcinogen to analyze the mechanism of carcinogenesis. It also induces developmental toxicity [21]. DMBA is biotransformed into an ultimate carcinogen, DMBA-3,4-diol-1,2-epoxide, by three sequential reactions mediated by CYPs and mEH [22,23]. In the mouse, CYP1B1 predominantly catalyzes the epoxidation of the 3,4-position of DMBA [24], and mEH hydrolyzes DMBA-3,4-epoxide to DMBA-3,4-diol. DMBA-3,4-diol is oxidized further to the ultimate carcinogen, DMBA-3,4-diol-1,2-epoxide, by CYP1A1 and CYP1B1. Recent results on the resistance to DMBA-induced carcinogenesis of mEH-null and CYP1B1-null mice indicate the involvement of CYP1B1 and mEH in mouse DMBA-induced carcinogenesis [19,25]. Thus, DMBA is suitable as a model compound to evaluate the role for embryonic activation in developmental toxicity.

In the present study, we conducted *in vivo* (embryo toxicity) and *in vitro* (cytotoxicity of embryo fibroblasts) experiments using mEH-null and wild-type mice to evaluate the role of embryonic and maternal mEH in DMBA-induced developmental toxicity. The results suggest that maternal mEH plays an important role in DMBA-induced developmental toxicity.

2. Materials and methods

2.1. Chemicals

DMBA was purchased from the Sigma Chemical Co. Dulbecco's modified Eagle's medium (DMEM) and penicillin–streptomycin were obtained from GIBCO BRL. DMBA-3,4-dihydrodiol (3,4-diol) was obtained from the NCI Chemical Carcinogen Repository. [*methyl*-^{3}H]Thy-^{3}H]Thymidine was obtained from Amersham Pharmacia Biotech.

2.2. Animals

mEH-null and wild-type mice were derived from a colony maintained by the National Institutes of Health [19]. These mouse lines were derived from the same parents backcrossed (twice) with C57BL/6 mice. Although the genetic background of these strains of mice is a mix between 129/SVJ and C57BL/6, both mouse lines possess a responsive type of Ah (aryl hydrocarbon) locus derived from C57BL/6 [26]. Mice were housed in plastic cages on hardwood bedding and were allowed food and tap water *ad lib.*

2.3. DMBA-induced developmental toxicity

Two or three virgin females (7- to 10-weeks-old) were placed in a cage with a male mouse. The presence of a vaginal plug was considered evidence of copulation, and putative pregnant females were removed and caged together (four to five females per cage). This was counted as GD 0. DMBA dissolved in dimethyl sulfoxide was diluted five times with water (2.5 mg/mL). Pregnant females were treated i.p. with DMBA (50 mg/kg) daily from GD 11 to GD 15. Control mice were treated with the vehicle. On GD 18, pregnant females were killed by cervical dislocation. Their uteri were removed, and the number of resorbed fetuses was noted. Each live fetus was weighed and measured from crown to rump.

2.4. Preparation of embryo fibroblasts

Embryo fibroblasts (EFs) were prepared as described previously [25]. Mice at GD 14 were killed, the embryos were placed in phosphate-buffered saline (pH 7.4), and their internal organs and heads were removed. The remaining torsos were minced and suspended in 0.25% trypsin for 40 min at 37°. The reaction was stopped by the addition of incubation medium (DMEM with 10% fetal bovine serum, 100 μg/mL of penicillin, 100 μg/mL of streptomycin, 250 μg/mL of amphotericin B, and 2 mM glutamine).

2.5. Cytotoxicity assay

The EF cells were cultured at 37° in an atmosphere of 5% carbon dioxide for 48 hr. The cells were trypsinized and seeded into 96-well plates at a density of 8×10^3/well in 100 μL of medium. Chemicals dissolved in 1 μL of dimethyl sulfoxide were added to 1 mL of medium. Following incubation for 24 hr, the medium containing the chemical was added to the cells, and incubation was continued for 72 hr. After the medium was removed, the cells were treated with neutral red solution (50 μg/mL) for 3 hr. After the neutral red solution was removed, the cells were fixed with a solution of 1% formalin containing 1% $CaCl_2$. The neutral red was extracted with 50% ethanol containing 1% acetic acid, and the absorbance at 540 nm was measured.

2.6. Cell proliferation assay

Cell proliferation was determined by [*methyl*-^{3}H]-thymidine incorporation. The cells were seeded into 96-well plates and treated with chemicals. [*methyl*-^{3}H]-Thymidine (1 μCi/mL) was added 48 hr after chemical

treatment. The cells were cultured for 2 hr, and 0.05 mL of 0.25% trypsin-EDTA was added. The plates were incubated for 10 min at 37° before harvesting by use of a cell harvester. Incorporated radioactivity was assessed in a Beckman LS 5000 scintillation counter.

2.7. Immunoblot analysis

Microsomal proteins were subjected to immunoblot analyses using anti-rat mEH antibody provided by Dr. James P. Hardwick (Northeastern Ohio University College of Medicine) and anti-rat CYP1B1 antibody provided by Daiichi Pure Chemicals.

2.8. Preparation of serum samples

Pregnant females were injected i.p. with DMBA (50 mg/kg) daily from GD 11 to GD 15. The mice were anesthetized with ether 5 hr after the final DMBA treatment, and blood was collected from the inferior vena cava. The blood was stored at room temperature for 30 min and centrifuged for 10 min at 10000 *g*. The supernatant (serum) was stored at −80°.

2.9. DMBA metabolism

DMBA metabolites were analyzed as described previously [27,28]. DMBA was re-crystallized from acetone/hexane. Incubation mixtures contained 1 mg of microsomal protein and DMBA (20 μM) in methanol (2.5 μL) in a final volume of 0.5 mL. The mixtures were preincubated at 37° for 2 min. To initiate the reaction, an NADPH-generating system was added to the reaction mixture. Incubations were carried out at 37° under subdued lighting for 20 min, and 2 mL of ethyl acetate/acetone (2:1) was added into the reaction mixtures to terminate the reaction and extract DMBA metabolites. DMBA metabolites in serum (0.5 mL) were also extracted with 2 mL of ethyl acetate/acetone (2:1). Pyrene methanol (2.5 pmol) was added as the internal standard. By comparing the retention time of the DMBA metabolites with the authentic compound, i.e. DMBA-3,4-dihydrodiol, the 3,4-diol metabolite was identified.

2.10. HPLC analysis

HPLC analyses were performed with a Jasco Intelligent model PV-980 pump and an FP-920S fluorescence detector. DMBA metabolites were separated with an ODS-AM column (250 × 4.6 mm) using a flow rate of 0.8 mL/min. The column was eluted with methanol/H_2O (1:1) for 10 min and then with a 50-min linear gradient of methanol/H_2O (1:1) to methanol (100%). The final elution with 100% methanol was continued for 10 min. DMBA metabolites were detected by using an excitation wavelength of 268 nm and emission wavelengths of 395 nm for the first 38 min, followed by 478 nm between 38 and 50 min, and then 415 nm between 50 and 70 min.

2.11. Statistical analysis

Data were analyzed with one-way factorial ANOVA and a post-hoc test (Scheffe test). All statistical tests were performed using the StatView-J 4.02, and a P value of less than 0.05 was considered significant.

3. Results

3.1. Developmental toxicity of DMBA in vivo

We previously reported that mEH-null mice were more resistant than wild-type mice to DMBA-induced skin carcinogenesis and spleen immunotoxicity [19,26]. These results suggest the possibility that mEH is involved in DMBA-induced developmental toxicity. In the present study, developmental toxicity tests were performed using mEH-null and wild-type mice and mating schemes that would generate mEH-null (−/−), heterozygous (+/−), and wild-type (+/+) embryos. First, the susceptibility to DMBA-induced developmental toxicity was compared between mEH-null and wild-type fetuses. Second, to evaluate the relative importance of maternal and embryonic mEH, DMBA-induced developmental toxicity was compared between mEH-null and heterozygous fetuses derived from mEH-null dams mated with mEH-null and wild-type males, respectively. If embryonic mEH is involved in DMBA-induced developmental toxicity, the heterozygous embryo is expected to be more susceptible to toxicity than the mEH-null embryo. As shown in Fig. 1, among wild-type, heterozygous and mEH-null fetuses from control (untreated) dams, there was no significant difference in weight and crown-rump length. On the other hand, in all matings, DMBA (50 mg/kg) treatment of the dams produced significant decreases in fetal weights and crown-rump lengths, compared with their controls. Wild-type fetuses from DMBA-treated dams showed a 31.1 and 15.1% decrease in weight and crown-rump length, respectively, compared to their controls. In contrast, the respective weights and crown-rump lengths of fetuses from DMBA-treated mEH-null dams were decreased 13.1 and 5.7% for mEH-null fetuses, and 10.5 and 6.6% for heterozygous fetuses. There were significant differences in weight ($P = 0.0009$) and crown-rump length ($P = 0.0003$) between wild-type and mEH-null fetuses from DMBA-treated dams, whereas no significant difference was found in weight and crown-rump length between mEH-null and heterozygous fetuses from DMBA-treated mEH-null dams (Fig. 1). In wild-type DMBA-treated dams, there was a 3.7-fold increase in fetal resorption compared with controls (Table 1).

To determine whether the wild-type dams were more susceptible to DMBA-induced toxicity than the mEH-null

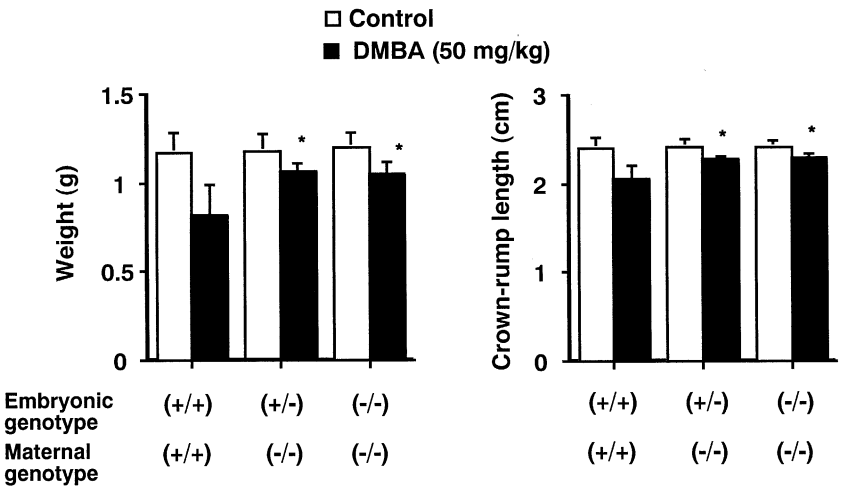

Fig. 1. Weight and crown-rump length of DMBA-treated mEH-null and wild-type mouse fetuses. Pregnant females were injected i.p. with DMBA (50 mg/kg) daily from GD 11 to GD 15. On GD 18, pregnant females were killed, and fetal weight and crown-rump length were measured. Values represent means ± SD (N = 8–13). Key: (*) significant difference from DMBA-treated wild-type mice ($P < 0.05$). (+/+), wild-type; (−/−), mEH-null; (+/−), heterozygote from the mating of wild-type mice with mEH-null mice.

dams, maternal organ weight was measured. Although no significant decrease was observed in the ratios of liver and kidney to body weights of wild-type and mEH-null dams treated with DMBA compared with control dams, spleen to body weight ratios decreased in both dams (data not shown). Furthermore, the spleen to body weight ratios of the wild-type dams were significantly lower than those of the mEH-null dams, suggesting that the wild-type dams were more susceptible to DMBA-induced spleen toxicity than were the mEH-null dams.

3.2. Cytotoxicity of DMBA in embryo fibroblasts

To analyze the mechanism of DMBA-induced toxicity, MEFs were used. For cell proliferation, no significant difference was detected in the doubling time between MEFs harvested from wild-type and mEH-null embryos. Cell numbers were increased 8-fold during 72 hr. To examine whether mEH is involved in DMBA-induced cytotoxicity, the effect of DMBA-treatment on MEFs isolated from wild-type and mEH-null embryos was compared. As shown in Fig. 2, treatment of wild-type MEFs with 3 μM DMBA produced a greater than 50% decrease in cell viability, whereas no significant decrease in viability was observed in mEH-null MEFs treated with the same concentration of DMBA.

DMBA-3,4-diol is a proximate carcinogen that is converted to the ultimate form, DMBA-3,4-diol-1,2-epoxide. To determine whether the DMBA-3,4-diol is involved in MEF toxicity, wild-type and mEH-null MEFs were treated with DMBA-3,4-diol. The DMBA-3,4-diol, at 0.3 μM, decreased the viability of both wild-type and mEH-null MEFs by almost 50% (Fig. 2). This diol caused a significant decrease in wild-type MEF viability at about a 10 times lower concentration than DMBA. Moreover, treatment with a 0.1 μM concentration or higher of DMBA-3,4-diol gave rise to cell killing. In contrast to the treatment with DMBA, no significant difference was observed in the susceptibility to DMBA-3,4-diol between wild-type and mEH-null MEFs.

Furthermore, the effect of DMBA or DMBA-3,4-diol treatment on the proliferation of wild-type MEFs was analyzed by [^{3}H]thymidine incorporation. Exposure to 0.01 μM DMBA-3,4-diol led to a 50% decrease in cell proliferation of wild-type MEFs (Fig. 3), whereas 0.1 μM DMBA produced only a 40% decrease, suggesting that the

Table 1
Reproductive outcome in mEH-null and wild-type mice treated with DMBA

Maternal genotype	Paternal genotype	Embryonic genotype	Dose (mg/kg)	Dams (N)	Inplants (N)	Live fetus (N)	Resorptions (%)
(+/+)	(+/+)	(+/+)	0	10	77	72	6.5
			50	8	58	44	24.1
(−/−)	(+/+)	(+/−)	0	13	112	104	7.1
			50	8	79	73	7.6
(−/−)	(−/−)	(−/−)	0	10	86	82	4.7
			50	11	88	79	10.2

(+/+), wild-type; (−/−), mEH-null; (+/−) heterozygote.

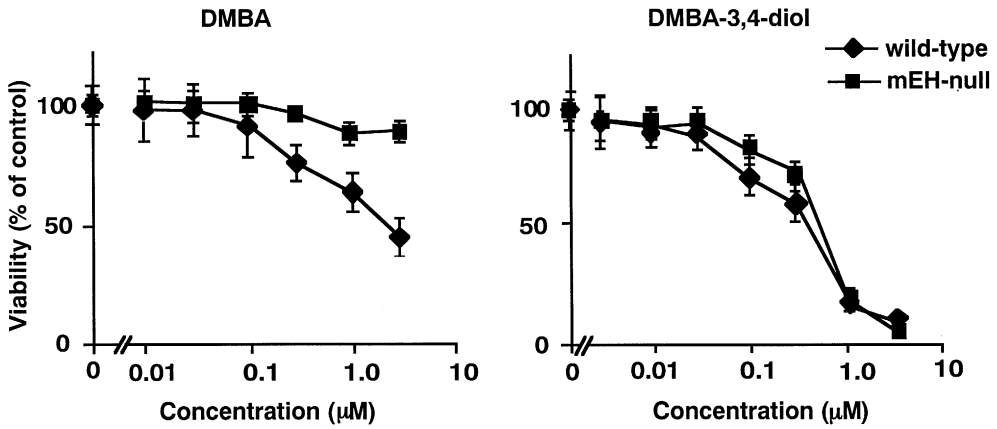

Fig. 2. Cytotoxicity of DMBA and DMBA-3,4-diol in embryo fibroblasts. Embryo fibroblasts were prepared from wild-type and mEH-null mouse embryos (GD 14). Cell viability was assessed by neutral red incorporation, after embryo fibroblasts were treated with DMBA or DMBA-3,4-diol for 72 hr. Data are expressed as a percentage of the vehicle control for each MEF isolate. Values represent means ± SD (N = 12).

DMBA-3,4-diol more potently inhibited proliferation of the wild-type MEFs than did DMBA.

3.3. Expression of drug-metabolizing enzymes

To evaluate the metabolic activation potency of DMBA in maternal and embryonic organs, expression levels of mEH and CYP1B1 were analyzed in maternal and embryonic livers and placentas from DMBA-treated dams. mEH was detected in the microsomal protein fraction of wild-type maternal and embryonic (GD 15) livers and placentas (Fig. 4). No significant difference in the expression level of the protein was observed in liver microsomes between wild-type and heterozygous mice [19]. On the other hand, CYP1B1 immunoreactivity was detected among the microsomal proteins from maternal placentas and embryonic livers of both mEH-null and wild-type mice. Reactivity to the anti-rat CYP1A1 antibody was also detected in the microsomal protein fraction from the livers of embryos from DMBA-treated dams (data not shown).

3.4. Detection of DMBA-3,4-diol

To evaluate the level of DMBA-3,4-diol formed in maternal and embryonic organs, microsomal DMBA metabolites from maternal and embryonic livers and placentas from DMBA-treated pregnant mice were analyzed. DMBA-3,4-diol was detected in microsomal metabolites from both maternal liver and placenta of wild-type mice, but not from embryonic liver under the same conditions (Fig. 5). DMBA-3,4-diol formation in maternal liver microsomes was 9.1 times higher than that in placental microsomes. Furthermore, the DMBA-3,4-diol was also detected among the microsomal DMBA metabolites from wild-type MEFs at a similar level as found in the maternal liver microsomes (data not shown). The DMBA-3,4-diol

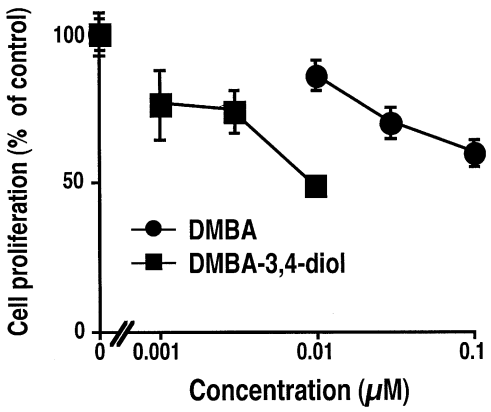

Fig. 3. [^{3}H]Thymidine incorporation by wild-type MEFs. Fibroblasts were prepared from mouse embryos (GD 14). The embryo fibroblasts (GD 14) were cultured with [^{3}H]thymidine for 2 hr following treatment with DMBA or DMBA-3,4-diol for 48 hr. The [^{3}H]thymidine incorporation was normalized to cell number (4×10^4 cells). Incorporated radioactivity was assessed in a Beckman LS 5000 scintillation counter. Data are expressed as a percentage of the vehicle control for each MEF isolate. Values represent means ± SD (N = 12).

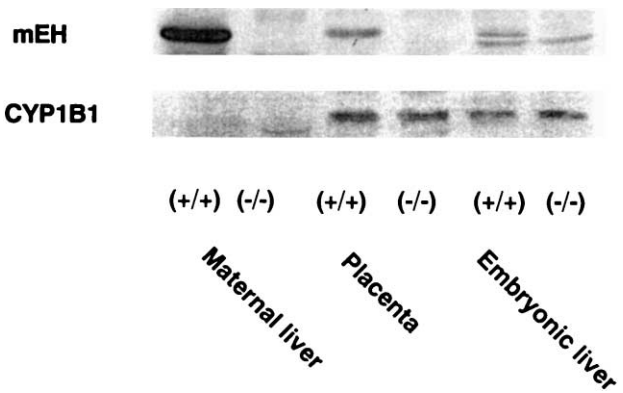

Fig. 4. Immunoblot analyses of mEH and CYP1B1. Microsomes were prepared from DMBA-treated pregnant (GD 15) mice. Each lane was loaded with 50 μg of microsomal protein. Mouse mEH and CYP1B1 proteins were detected with anti-rat mEH antibody and anti-rat CYP1B1 antibody, respectively. This immunoblot is representative of three experiments. (+/+), wild-type mice; (−/−), mEH-null mice.

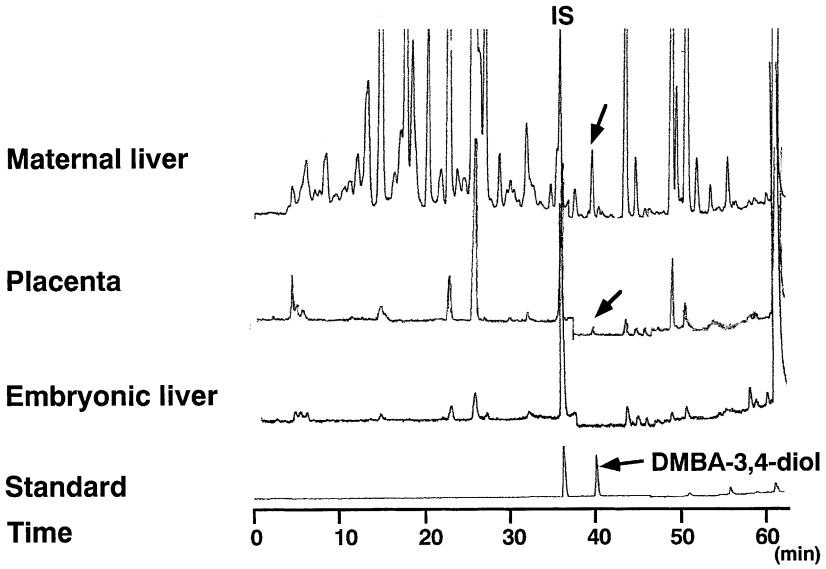

Fig. 5. HPLC profile of microsomal DMBA metabolites in maternal and embryonic livers and placentas of wild-type mice. The chromatograms represent metabolic profiles obtained from a 20-min incubation of DMBA with each microsomal preparation (1 mg) from DMBA-treated mice. IS represents the pyrene methanol peak used as an internal standard.

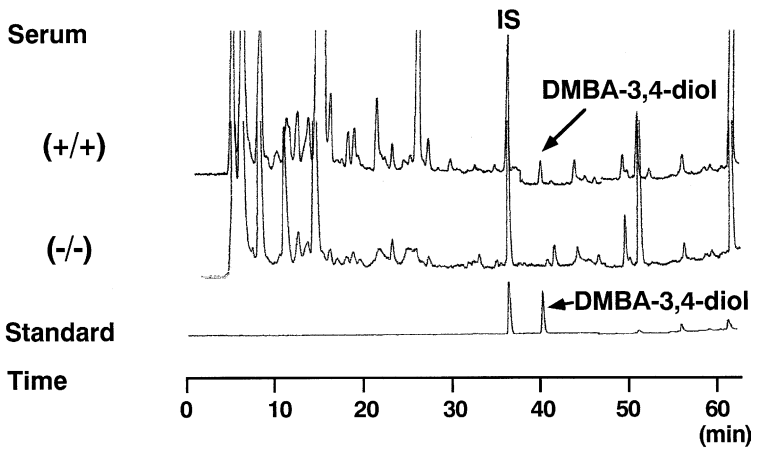

Fig. 6. HPLC profile of DMBA metabolites in sera from wild-type and mEH-null DMBA-treated pregnant mice. The chromatograms represent metabolic profiles obtained from sera of DMBA-treated (50 mg/kg) pregnant mice (GD 15). IS represents the pyrene methanol peak as an internal standard. (+/+), wild-type mice; (−/−), mEH-null mice.

was not detected among the microsomal metabolites from mEH-null mice (data not shown). Maternal organs of the wild-type mice produced the highest levels of the DMBA-3,4-diol. Therefore, sera from wild-type and mEH-null DMBA-treated mice were subjected to HPLC analysis for the detection of the DMBA-3,4-diol. As shown in Fig. 6, DMBA-3,4-diol was detected in sera from wild-type pregnant mice treated with DMBA, but not from mEH-null pregnant mice.

4. Discussion

mEH is known to play a key role in the metabolic activation of DMBA to its ultimate carcinogen, DMBA-3,4-diol-1,2-epoxide. DMBA is converted metabolically to this reactive electrophilic epoxide by CYPs and mEH. In the present study, *in vivo* and *in vitro* experiments were carried out using mEH-null and wild-type mice to evaluate the role of embryonic mEH in DMBA-induced developmental toxicity.

In vivo studies revealed that mEH-null embryos from mEH-null dams were more resistant to DMBA-induced developmental toxicity than the wild-type embryos from wild-type dams. No significant difference, however, was observed in the susceptibility of DMBA-induced toxicity between mEH-null embryos and heterozygous embryos from mEH-null dams. These results suggest that DMBA-induced developmental toxicity is dependent upon the mEH genotype of the dams, not that of the embryos. These data are consistent with a major role of maternal mEH in DMBA-induced embryo toxicity.

In vitro experiments demonstrated that mEH-null MEFs were more resistant to DMBA-induced cytotoxicity than wild-type MEFs. Furthermore, the DMBA metabolite, DMBA-3,4-diol, suppressed MEF proliferation and was more cytotoxic than DMBA to both mEH and wild-type MEFs. No significant difference was observed in the susceptibility of mEH-null and wild-type MEFs to DMBA-3,4-diol-induced cytotoxicity. These results suggest that the mEH-mediated production of the DMBA-3,4-diol is a critical step for DMBA-induced toxicity and a critical determinant for the susceptibility of DMBA-induced toxicity. Although we did not analyze the mechanism for DMBA-3,4-diol-induced cytotoxicity, it is proposed that the DMBA-3,4-diol metabolite might bind proteins and lipids, resulting in the modification of molecules which then cause lipid peroxidation and alterations of enzyme and membrane functions.

Immunoblot analyses showed that mEH protein was expressed in embryonic livers of GD 15 (Fig. 4). These results are consistent with a previous report that described the detection of the mEH protein and its associated enzymatic activity in human embryos at GD 53 [10]. GD 15 of mice corresponds to approximately GD 58–60 of humans embryologically [29]. As shown in Fig. 4, the immunoblot analyses indicate the possibility that the embryo at GD 15 has the potential to produce the DMBA-3,4-diol. However, the DMBA-3,4-diol was not detected among the microsomal DMBA metabolites of wild-type embryonic liver (GD 15), although those of placenta from wild-type mice produced a small amount of the DMBA-3,4-diol (Fig. 5). Because the levels of metabolic activity appear low in embryonic liver, it is unlikely that DMBA transported to the embryo is metabolized to the ultimate toxic compounds by embryonic CYPs and mEH during organogenesis. The finding of the DMBA-3,4-diol in sera of wild-type pregnant mice treated with DMBA suggests the possibility that the DMBA-3,4-diol produced by maternal CYPs and mEH is transported to the embryo and that the DMBA-3,4-diol was further oxidized to the ultimate toxic compounds within the embryo. This hypothesis is consistent with the results of *in vivo* and *in vitro* toxicity experiments.

Immunoblot analyses of mEH detected a second protein band appearing in the wild-type embryonic liver lane (Fig. 4). This band also was found in the mEH-null embryonic liver lane. Several protein bands cross-reacting with the antibody against rat mEH were detected in embryonic liver microsomes under our experimental conditions. We could not identify this protein. Immunoblot analyses of CYP1B1 showed that the expression level of CYP1B1 protein was very low. Our previous report demonstrated that CYP1A1 and CYP1A2 are expressed in liver microsomes from DMBA-treated mice [26]. These results suggest the possibility that the DMBA-3,4-diol detected in maternal liver microsomes and sera is produced by CYP1A2 and/or CYP1A1. Our results are consistent with previous reports [28,30].

In the present study, we could not distinguish between direct (embryonic) and secondary (maternal) effects of DMBA-induced toxicity on the embryos. Maternal DMBA toxicity was detected more clearly in the spleens of wild-type dams than of mEH-null dams. In addition, we cannot exclude the possibility that the DMBA-induced toxicity in the embryo is not secondary to maternal toxicity. Furthermore, lower, but significant DMBA-induced toxicity was observed in mEH-null embryos. These results indicate the existence of a metabolic activation pathway independent of mEH [31] or a mechanism that is independent of the metabolic activation of DMBA [32,33].

In the present study, we established that maternal mEH but not embryonic mEH is a critical determinant for susceptibility to DMBA-induced developmental toxicity. Although our experiments provided no evidence for the involvement of embryonic mEH in DMBA-induced developmental toxicity, further experiments are necessary to identify the role of embryonic mEH in developmental toxicity induced by drugs such as phenytoin and environmental chemicals.

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