Involvement of Microsomal Epoxide Hydrolase Enzyme in Ovotoxicity Caused by 7,12-Dimethylbenz[a]anthracene

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Ovarian follicle disruption in mice caused by 7,12-dimethylbenz[a]anthracene (DMBA) is attributed to its bioactivation by CYP1B1 to a 3,4-epoxide which is then hydrolyzed to form a 3,4-diol by microsomal epoxide hydrolase (mEH). Further epoxidation by CYP1A1 or 1B1 forms the ultimate ovotoxicant, DMBA-3,4-diol-1,2-epoxide. Studies suggest that the mouse ovary expresses these enzymes, and thus, may be capable of bioactivating DMBA to its ovotoxic metabolite. The present study was designed to evaluate the role of ovarian mEH in DMBA-induced ovotoxicity using a novel neonatal mouse ovarian culture system. Ovaries from postnatal day (PND) 4 B6C3F1 mice were incubated with DMBA (12.5nM–1 μM) for various lengths of time. Following incubation, ovaries were histologically evaluated or assessed for mEH protein or mRNA. Following 15 days of incubation, DMBA reduced (p < 0.05) healthy follicles at concentrations ≥ 12.5nM. At 1μM DMBA, follicle loss and increased mEH protein were measured (p < 0.05) by 6 h. mRNA encoding mEH markedly increased after 2 days of incubation, and this increase preceded accelerated follicle loss at 4 days. Furthermore, follicle loss induced by DMBA was prevented when cyclohexene oxide (2mM), an mEH inhibitor, was added to DMBA incubations. These studies suggest that the PND4 mouse ovary is capable of bioactivating DMBA to its ovotoxic form, and that ovarian mEH enzyme activity is likely involved. Furthermore, these observations support the use of a novel ovarian culture system to study ovary-specific metabolism of xenobiotic chemicals.

Key Words: DMBA; mEH; in vitro ovarian culture; ovary.

The polycyclic aromatic hydrocarbon, 7,12-dimethylbenz[a]anthracene (DMBA) is a widely studied model carcinogen for the induction of mammary (Russo and Russo, 1996), ovarian (Kanter et al., 2006), and skin (Diagaradjane et al., 2006) tumors in rodents. Sources of human exposure to this compound are cigarette smoke, car exhaust, and burning of other organic substances such as coal, oil, wood, and rubbish. It has also been shown that DMBA causes ovarian follicle disruption and ovarian failure in mice. DMBA targets all follicle populations and corpora lutea in a dose-dependent manner, leading to a decrease in ovarian volume (Mattison, 1980; Weitzman et al., 1992).

Both the carcinogenic property and ovarian effects of this compound are attributed to the bioactivation of DMBA to a 3,4-diol-1,2-epoxide metabolite. DMBA is bioactivated by cytochrome P450 (CYP450) isofrom 1B1 to a 3,4-epoxide, which is then hydrolyzed to form a 3,4-diol by the microsomal epoxide hydrolase (mEH) enzyme. This compound further undergoes epoxidation by CYP450 isoforms CYP1A1 or 1B1 to form the ultimate ovotoxicant and carcinogen, 3,4-diol-1,2-epoxide (Fig. 1; Savas et al., 1997; Shimada et al., 2001).

Results of studies with transgenic mice provide additional evidence for the role of CYP450 and mEH in the bioactivation of DMBA, leading to toxicity. CYP1B1 null mice treated with DMBA were less susceptible to DMBA-DNA adduct formation in the thymus, spleen, ovary, and the testis (Buters et al., 2003). Miyata et al. (1999) reported that mEH null mice treated with DMBA were less susceptible to incidents of skin cancer. In addition, the 3,4-diol-DMBA metabolite was detected neither in DMBA-treated mEH null mouse embryo fibroblast cultures nor in serum of mEH null mice (Miyata et al., 1999, 2002). 3,4-diol-DMBA has been shown to be a more potent ovarian toxicant compared to that of the parent compound (Matikainen et al., 2001).

Studies have shown that the mouse ovary expresses CYP1A1, CYP1B1, and mEH, and mRNA encoding these enzymes is inducible following dosing with substrates for these enzymes (Cannady et al., 2002; Shimada et al., 2003). Inhibition of rat ovarian CYP1B1 activity using an anti-P4501B1 IgG markedly reduced DMBA metabolism (Otto et al., 1992). Furthermore, mEH enzyme activity in the mouse ovary was induced following in vivo dosing with the industrial chemical 4-vinylcyclohexene diepoxide, VCD, which is detoxified by mEH (Cannady et al., 2002). mEH protein expression was detected in human granulosa, theca interna, and luteal cells, and catalytic activity was detected in granulosa cells and microsomal fractions from corpora lutea (Hattori et al., 2000). Unilateral intraovarian injection of mice with
DMBA destroyed follicles only in the treated ovary (Shiromizu and Mattison, 1985). Studies have shown that DMBA can be metabolized by ovarian and placental microsomes (Bengtsson et al., 1983; Miyata et al., 2002). Furthermore, studies by Becedas et al. (1993) suggest that rat granulosa cells in culture can metabolize DMBA to the carcinogenic, 3,4-diol-1,2-epoxide. Collectively, these findings suggest that the ovary is capable of bioactivating DMBA to the 3,4-diol-1,2-epoxide.

Even though studies indicate that the ovary is capable of bioactivating DMBA to the ovotoxicant, the role of ovarian enzymes, such as mEH, in the resulting toxicity of DMBA has not been assessed. Therefore, the present study was designed to evaluate the role of ovarian mEH in DMBA-induced ovotoxicity utilizing a novel in vitro ovarian culture system. Using this system, hepatic contribution to bioactivation of DMBA is removed, and ovary-specific capabilities can be assessed. The hypothesis is that ovarian mEH is involved in bioactivation of DMBA to the 3,4-diol-1,2-epoxide as evidenced by ovarian toxicity.

MATERIALS AND METHODS

Reagents. DMBA, cyclohexene oxide (CHO), bovine serum albumin (BSA), ascorbic acid (vitamin C), and transferrin were purchased from Sigma-Aldrich Inc (St Louis, MO). Dulbecco’s Modified Eagle Medium: nutrient mixture F12 (Ham) 1X (DMEM/Ham’s F12), Albumax, penicillin/streptomycin (5000 U/ml, 5000 µg/ml, respectively), Hanks’ Balanced Salt Solution (without CaCl2, MgCl2, or MgSO4), mEH and β-actin custom-designed primers, and Superscript III One-Step RT-PCR System were obtained from Invitrogen Co. (Carlsbad, CA). Millicell-CM filter inserts were purchased to cover the top of the ovary to prevent drying. Ovaries were incubated with 1% DMSO (vehicle control), DMBA, and/or CHO at concentrations indicated in figure legends. One micromolar DMBA concentration was adapted from Matikainen et al. (2001). Plates containing ovaries were cultured at 37°C and 5% CO2 in air. For those cultures lasting more than 2 days, media were removed and fresh media and treatment were replaced every 2 days. Four culture studies (three to five ovaries per treatment per culture) were conducted per experiment.

Histological evaluation of follicle numbers. Following incubation, ovaries were placed in Bouin’s fixative for 1.5 h, transferred to 70% ethanol, embedded in paraffin, serially sectioned (5 µm thick), and every sixth section was mounted. All ovarian sections were stained with hematoxylin and eosin. Healthy follicle populations containing oocytes were classified and counted in every 12th section. Unhealthy follicles were distinguished from healthy follicles by pyknosis of granulosa cells and intense eosinophilic staining of oocytes (Devine et al., 2002a). Follicle population classification was according to the procedure of Flaws et al. (1994) which was adapted from that described by Pedersen and Peters (1968). Briefly, primordial follicles contained the oocyte surrounded by a single layer of squamous-shaped granulosa cells. Primary follicles contained the oocyte surrounded by a single layer of cuboidal-shaped granulosa cells, and secondary follicles contained the oocyte surrounded by multiple layers of granulosa cells. Total follicle loss for Figure 5 was calculated by subtracting the total number of follicles (primordial + primary + secondary) remaining following DMBA treatment at each time point from total number of follicles in vehicle control–treated group at that time point. These values were divided by the follicle numbers present in control ovaries, and the resulting value was multiplied by 100 to obtain a percentage, (Control − DMBA)/Control × 100.

Toluidine blue staining. This histological method was adapted from Tome et al. (2001). Briefly, following incubation, ovaries were fixed in 3% glutaraldehyde in 0.1M cacodylate (pH = 7.2) for 1.5 h, transferred to 1mM cacodylate, embedded in epoxy resin, serially sectioned (1 µm thick), and every 10th section was mounted. All ovarian sections were stained with toluidine blue for observation of pyknotic nuclei as a marker for apoptosis.

RNA isolation. Following 3 h, 6 h, 24 h, and 2 days of in vitro culture, ovaries (12 per pool) treated with vehicle control (1% DMSO) or DMBA (1µM) were stored in RNAlater at − 80°C. Total RNA was isolated using RNeasy Mini kit. Briefly, ovaries were lysed and homogenized using a motor pestle followed by applying the mixture onto a QIAshredder column. The QIAshredder column containing ovarian tissue sample was then centrifuged at 14,000 rpm for 2 min. The resulting supernatant was applied to an RNeasy mini column, allowing the RNA to bind to the filter cartridge. Following washing, the RNA was eluted from the filter and concentrated using RNeasy MinElute kit. Briefly, isolated RNA was applied to an RNeasy MinElute spin column, and after washing, RNA was eluted using 14 µl of RNase-free water. RNA concentration was determined using a NanoDrop (λ = 260/280 nm; ND 1000).

First strand cDNA synthesis and real-time PCR. Total RNA (1 µg) was reverse transcribed into cDNA utilizing the Superscript III One-Step RT-PCR system, hepatic contribution to bioactivation of DMBA is removed, and ovary-specific capabilities can be assessed. The hypothesis is that ovarian mEH is involved in bioactivation of DMBA to the 3,4-diol-1,2-epoxide as evidenced by ovarian toxicity.

Animals. Late gestation day pregnant mice (carrying B6C3F1 litters) were purchased from Harlan Laboratories (Indianapolis, IN). All animals were housed one per cage in plastic cages, and maintained in a controlled environment (22 ± 2°C; 12-h light/12-h dark cycles). The animals were provided with a standard diet with ad libitum access to food and water and allowed to give birth. All animal experiments were approved by the University of Arizona’s Institutional Animal Care and Use Committee.

In vitro ovarian cultures. Postnatal day (PND) 4 female B6C3F1 mice were killed by CO2 inhalation followed by decapitation. Each ovary was removed, oviduct and excess tissue trimmed, and placed on a piece of Millicell-CM membrane floating on 250 µl of DMEM/Ham’s F12 medium containing 1 mg/ml BSA, 1 mg/ml Albumax, 50 µg/ml ascorbic acid, 5 U/ml penicillin/5 µg/ml streptomycin, and 27.5 µg/ml transferrin in a well in a 48-well plate previously equilibrated to 37°C. Using fine forceps, a drop of medium was placed to cover the top of the ovary to prevent drying. Ovaries were incubated with 1% DMSO (vehicle control), DMBA, and/or CHO at concentrations indicated in figure legends. One micromolar DMBA concentration was adapted from Matikainen et al. (2001). Plates containing ovaries were cultured at 37°C and 5% CO2 in air. For those cultures lasting more than 2 days, media were removed and fresh media and treatment were replaced every 2 days. Four culture studies (three to five ovaries per treatment per culture) were conducted per experiment.
RESULTS

Effect of Concentration on DMBA-Induced Follicle Loss

Follicle loss was evaluated in PND4 mouse ovaries following 15 days of incubation with various concentrations (12.5nM–1μM) of DMBA (Fig. 2). Compared to vehicle control, DMBA reduced (p < 0.05) healthy primordial follicles at concentrations ≥ 12.5nM (Fig. 2A). Healthy primary follicles were reduced (p < 0.05) at concentrations ≥ 25nM DMBA (Fig. 2B). DMBA markedly reduced (p < 0.05) healthy secondary follicles at all concentrations (Fig. 2C). All healthy follicle populations were depleted by DMBA at concentrations ≥ 250nM.

Time Course of DMBA-Induced Follicle Loss

Follicle loss was evaluated in PND4 mouse ovaries incubated with 1μM DMBA for various time points in culture (Fig. 3). Relative to vehicle control, ovaries incubated with DMBA for 6 h, showed healthy primordial and primary follicle loss (p < 0.05). All healthy primordial and primary follicles were depleted in these ovaries by 8 days of culture (Figs. 3A and 3B). Secondary follicles in PND4 ovaries do not develop until 4 days. No healthy secondary follicles were observed in DMBA-treated ovaries at any time point (Fig. 3C). However, some unhealthy secondary follicles in DMBA-treated ovaries were observed between 4 and 15 days of culture (data not shown).

DMBA-Induced Cell Death

The type of cell death occurring in cultured ovaries following incubation with DMBA (4 days) was evaluated using toluidine blue staining (Fig. 4). Ovaries treated with DMBA contained
pyknotic bodies in both the oocyte and granulosa cell nuclei. Some follicles in these ovaries also contained vacuoles along with pyknotic bodies (Figs. 4C and 4D). These morphological changes are characteristic of apoptosis (Kerr et al., 1972; Tome et al., 2001). Follicles containing pyknotic bodies that were rarely observed in control-treated ovaries were assumed to be undergoing the normal atretic process (cell death by apoptosis).

**Effect of DMBA on Ovarian Expression of mEH mRNA**

To investigate the effect of DMBA on ovarian mEH enzyme expression, the level of mEH mRNA in ovaries collected from PND4 mice following DMBA treatment was quantified using real-time PCR (Fig. 5A). mEH mRNA was detected in RNA isolated from ovaries in all treatment groups at all time points. Following incubation with 1μM DMBA for 3, 6, or 24 h, the level of mEH mRNA did not change compared to that of the vehicle control–treated group. At 2 days, there was a 5.2-fold increase (p<0.05) in mEH mRNA compared to vehicle control (Fig. 5A). Following 2 days of DMBA treatment, very few healthy follicles were observed; thus, not enough RNA could be isolated from ovaries to conduct real-time PCR reactions following this time point.

Figure 5B shows the total follicle loss expressed as a percentage throughout the 15-day DMBA incubation period. The rate of DMBA-induced follicle loss from 6 h to 2 days did not differ significantly. At 4 days, the rate of DMBA-induced follicle loss increased (p<0.05), at which point it plateaued until 15 days of culture (Fig. 5B).

**Effect of DMBA on Ovarian Expression of mEH Protein**

To further evaluate the ovarian distribution of mEH, protein expression was visualized using confocal microscopy in ovaries collected from PND4 mice (Fig. 6). mEH protein was detected in all follicle populations present in PND4 ovaries (primordial and primary). Staining intensity for mEH in these follicles was highly localized to the oocyte cytoplasm. mEH protein was not detected in granulosa cells of either primordial or primary follicles (Figs. 6A and 6B). Following incubation with 1μM DMBA for 6 h, mEH staining in primary oocytes increased (p<0.05) compared to that of vehicle control ovary (Figs. 6C,
Interestingly, some diffuse mEH staining was detected in the primary oocyte nucleus in these DMBA-treated ovaries. There was no difference in staining intensity for mEH expression in primordial follicles between DMBA-treated and vehicle control ovaries (Fig. 6F). No Cy-5 staining was seen in immunonegative sections at $\lambda = 647$ nm (Fig. 6E).

**Effect of mEH Inhibitor on DMBA-Induced Follicle Loss**

Follicle loss was evaluated in PND4 mouse ovaries following 6 h of incubation with an mEH enzyme inhibitor, CHO (Fig. 7). CHO acts as an alternative substrate for mEH, and competitively inhibits mEH activity. Thus, metabolism of DMBA to the 3,4-diol-1,2-epoxide (active metabolite) is decreased (Oesch, 1973). CHO (2mM) did not affect primordial and primary follicle populations following 6 h of incubation. DMBA significantly ($p < 0.05$) decreased primordial and primary follicles following 6 h in culture. Loss of primordial and primary follicles was prevented by the addition of 2mM CHO to DMBA incubations (Figs. 7A and 7B).

**DISCUSSION**

In addition to having carcinogenic properties, DMBA has been shown to be an ovarian toxicant. DMBA targets all follicle populations in the ovary following in vivo dosing leading to ovarian failure (Mattison, 1980; Weitzman et al., 1992). Previous studies indicate that DMBA metabolism to the 3,4-diol-1,2-epoxide is required for carcinogenic and ovarian toxic effects of this compound (Sawicki et al., 1983; Shiromizu and Mattison, 1985; Vigny et al., 1985). Metabolic activation of DMBA to 3,4-diol-1,2-epoxide requires three sequential bioactivation steps mediated by CYP1B1, mEH, and CYP1A1/1B1 (Savas et al., 1997; Shimada et al., 2001). Although the liver is the primary organ participating in bioactivation of xenobiotics, extrahepatic organs such as the ovary have also been shown to be capable of bioactivation of xenobiotic chemicals. Both CYP1A1 and CYP1B1 are expressed in the ovary and mRNA for these CYP450s was induced in the ovary following a single dose of DMBA (Shimada et al., 2003). mEH enzyme is expressed in the ovary, and following dosing with the ovarian toxicant 4-vinylcyclohexene diepoxide, VCD, mEH activity, was induced in VCD-targeted follicle populations (Cannady et al., 2002). The study reported here provides
follicles counted per ovary, (B) primary follicles were classified and counted. Values are mean ± SE total described in the “Materials and Methods” section. Healthy (A) primordial and incubation, ovaries were collected and processed for histological evaluation as CHO, 1 l DMBA, or 1μM DMBA + 2mM CHO for 6 h. Following incubation, ovaries were collected and processed for histological evaluation as described in the “Materials and Methods” section. Healthy (A) primordial and (B) primary follicles were classified and counted. Values are mean ± SE total follicles counted per ovary, n = 5, *p < 0.05.

FIG. 7. Effect of mEH inhibitor (CHO) on DMBA-induced follicle loss. Ovaries from PND4 B6C3F1 mice were cultured with vehicle control, 2mM CHO, 1μM DMBA, or 1μM DMBA + 2mM CHO for 6 h. Following incubation, ovaries were collected and processed for histological evaluation as described in the “Materials and Methods” section. Healthy (A) primordial and (B) primary follicles were classified and counted. Values are mean ± SE total follicles counted per ovary, n = 5, *p < 0.05.

additional evidence for the role of mEH in chemical-induced ovarian toxicity. Even though studies suggest that the ovary can metabolize DMBA, the role of ovarian metabolism in resulting ovotoxicity has not been evaluated in the absence of hepatic contributions.

Previous studies have shown that rat PND4 ovaries can be cultured up to 15 days utilizing an in vitro culture method (Devine et al., 2002a,b). Rat PND4 ovaries in culture remain healthy and retain their dynamic characteristics. For example, PND4 ovary does not contain secondary follicles; however, following 15 days of in vitro culture, follicles develop to the secondary stage. Furthermore, this culture system has been utilized to study the mechanism of ovotoxicity induced by the ovarian toxicant VCD. As with in vivo dosing, following 15 days of exposure, VCD selectively destroyed primordial and primary follicles via apoptosis in cultures of PND4 ovaries (Devine et al., 2002b, 2004). Thus, the in vitro ovarian cultures mimic VCD-induced follicle loss seen with in vivo dosing studies. Therefore, the current study was designed to evaluate a possible role of ovarian mEH in metabolism of DMBA using this novel ovarian culture system. By using this approach, the role of ovarian metabolism of DMBA and DMBA-induced ovotoxicity can be evaluated, independent of hepatic contributions.

In PND4 rat ovarian cultures, following 15 days of VCD exposure, unhealthy degenerating follicles as well as healthy follicles were observed. Unhealthy follicles were characterized by pyknosis of granulosa cells and intense eosinophilic staining of the oocytes (Devine et al., 2002a). In the present study, DMBA reduced healthy primordial and secondary follicles at concentrations ≥ 12.5nM following 15 days of incubation. Unlike primordial and secondary follicles, primary follicle loss was observed at concentrations ≥ 25nM. This observation is interesting because VCD also had less of an effect on primary follicles compared to that of primordial follicles in PND4 rat ovarian cultures (Devine et al., 2004). DMBA ≥ 250nM depleted all healthy ovarian follicles in culture following 15 days of incubation. At that time, follicles in ovaries incubated with DMBA had lost their shape, burst, and become part of the interstitial space (data not shown). A similar degree of ovotoxicity was seen in PND4 B6C3F1 ovaries incubated with 30μM VCD (15 days; Rajapaksa, Cannady, Sipes and Hoyer, unpublished data). Therefore, DMBA (250nM) induces ovotoxicity in the culture system at a much lower concentration compared to VCD (30μM). This greater potency of DMBA compared with VCD was also observed in an in vivo 15-day dosing study, where an equivalent degree of follicle loss (ED50) in mice was seen with 0.02 mg/kg DMBA compared with 80 mg/kg for VCD (Borman et al., 2000).

The highest concentration of DMBA (1μM) was then utilized to determine the shortest time point for follicle loss, to be used in studies for evaluating the role of mEH. Ovaries were incubated with 1μM DMBA or vehicle. Following 6 h in culture, DMBA decreased healthy primordial and primary follicles. Secondary follicles in PND4 ovaries begin to form after 4 days in culture. No healthy secondary follicles were observed in ovaries cultured with DMBA, at any time point. However, unhealthy secondary follicles were observed in DMBA-treated ovaries cultured ≥ 4 days. Therefore, DMBA targeted secondary follicles directly, rather than preventing recruitment from the primary to secondary stage. By 8 days, all healthy ovarian follicle populations were depleted in ovaries incubated with DMBA.

Ovarian toxicants such as VCD have been shown to induce follicle loss in rats via acceleration of the natural process of atresia (apoptosis; Springer et al., 1996a,b; Hu et al., 2001a,b). Therefore, to further examine the type of cell death caused by DMBA, ovaries were stained with toluidine blue following 4 days in culture. In ovaries treated with DMBA (1μM), pyknotic bodies were detected in granulosa cells and oocytes of ovarian follicles, while other follicles contained pyknotic bodies along with vacuoles. These morphological changes are characteristic of apoptosis (Kerr et al., 1971; Tome et al., 2001). Thus, it can be hypothesized that DMBA induces ovarian follicle death via apoptosis. Previous studies by Matikainen et al. (2001) have shown an increased expression of Bax (proapoptotic member of the Bcl-2 family of proto-oncogenes) protein in primordial and primary follicles in ovaries incubated with DMBA in culture. Furthermore, follicle loss was not observed in Bax null ovaries cultured with DMBA.
compared to wild-type ovaries. Thus, collectively the results of these studies suggest that, as with other ovotoxicants, DMBA-induced follicle loss is via an apoptotic-dependent mechanism. Toluidine blue staining only affords a morphological assessment. Studies are currently underway to mechanistically evaluate DMBA-induced apoptosis using molecular approaches.

An evaluation was made as to whether follicle loss induced by DMBA involves ovarian mEH. Previous studies have shown that the adult B6C3F1 mouse ovary expresses catalytically active mEH, and this activity can be induced in the ovary following administration of VCD (Cannady et al., 2002). Therefore, expression of mEH protein in the PND4 ovary was evaluated by confocal microscopy. mEH was seen to be expressed in all follicle populations of the PND4 ovary. Expression was highly concentrated in oocyte cytoplasm. Unlike the adult B6C3F1 mouse ovary, mEH expression was not observed in PND4 ovarian granulosa cells (Cannady et al., 2002). Following DMBA treatment for 6 h, mEH expression increased in oocytes of primary but not primordial follicles. This is inconsistent with the observation that primordial follicles were more sensitive to DMBA-induced toxicity than primary follicles. Thus, it must be assumed that even though DMBA is more highly bioactivated in primary follicles, DMBA can diffuse into other ovarian compartments such as the primordial follicle pool. Conversely, the basal level of mEH expressed in primordial follicles may be sufficient to effectively bioactivate DMBA, to cause localized ovotoxicity.

Even though ovotoxicity and increased mEH protein expression were observed by 6 h following DMBA treatment, mRNA encoding mEH was not markedly increased until 2 days in culture. Previous studies have shown an increase in mEH protein level that could not be explained by an increase in gene transcription, and thus, mEH is thought to be also regulated post-transcriptionally (Kim and Kim, 1992; Simmons et al., 1987). It is also possible that this discrepancy is due to a decrease in protein degradation between the 6-h and 2-day time points. Interestingly, the rate of follicle loss marked increased between 2 and 4 days. Therefore, the induction of expression of mEH mRNA directly precedes a significant increase in the rate of follicle loss at 4 days. The lag in time between the marked increases in mRNA (2 days) for mEH and follicle loss (4 days) likely reflects the time between mRNA translation, mEH-stimulated bioactivation of DMBA, and onset of follicle loss.

CHO, an mEH inhibitor, was used to block DMBA bioactivation mediated by mEH. Incubation of ovaries with CHO alone for 6 h did not affect follicle populations. However, at that time DMBA induced follicle loss (primordial and primary, p < 0.05). Loss of follicles induced by DMBA was inhibited by coinubcation of ovaries with CHO. This observation provides functional support that ovarian mEH plays a role in bioactivation of DMBA.

In summary, data presented here suggest that DMBA is a highly potent ovarian toxicant. In these ovarian cultures, and most likely in vivo, ovarian mEH plays a key role in the bioactivation of DMBA. Ovarian mEH can be induced by DMBA at the transcriptional and translational levels. Thus, DMBA-induced expression of mEH appears to contribute to the high level of potency of DMBA. These findings support an extrahepatic role for target organ metabolism of xenobiotic agents that could amplify potential hepatic effects. Additionally, this study demonstrates that the in vitro PND4 whole ovary culture system will be useful for investigating ovarian capabilities for metabolism of xenobiotic chemicals.

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