ACADEMIC PRESS

Biochemical and Biophysical Research Communications 294 (2002) 101–107

BBRC

www.academicpress.com

Benzo(*a*)pyrene, but not 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, alters cell adhesion proteins in human uterine RL95-2 cells☆

Michelle A. McGarry,[a] Grantley D. Charles,[a,1] Theresa Medrano,[a] Michael R. Bubb,[b,c] Maria B. Grant,[a] Martha Campbell-Thompson,[b] and Kathleen T. Shiverick[a,*]

[a] *Department of Pharmacology and Therapeutics, University of Florida, P.O. Box 100267, JHMHSC, 1600 S.W. Archer Road, Gainesville, FL 32610-0267, USA*

[b] *Department of Medicine, College of Medicine, University of Florida, Gainesville, FL 32610, USA*

[c] *Malcolm Randall VAMC, College of Medicine, University of Florida, Gainesville, FL 32610, USA*

Received 25 April 2002

Abstract

This study compared the effects of benzo(*a*)pyrene (BaP) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), two aryl hydrocarbon receptor agonists, on cell attachment and adherens junction proteins in RL95-2 human uterine endometrial cells. Exposure to 10 μM BaP significantly decreased cell attachment to Matrigel, whereas 10 nM TCDD had no effect. Immunocytochemistry and Western immunoblot analysis showed that BaP, but not TCDD, produced a marked loss of plasma membrane epidermal growth factor receptor (EGF-R) localized along intercellular boundaries. BaP-treated cells exhibited significant decreases in β-catenin and cadherin protein levels, while vinculin levels remained unchanged relative to control. In contrast, TCDD treatment had no effect on the levels of β-catenin, cadherin, or vinculin. Further studies using the fluorescein labeled peptide phalloidin showed the presence of continuous subcortical actin filaments in control cells, whereas BaP-treated cells had subcortical actin aggregates. Thus, in contrast to TCDD, BaP produces a loss of cell attachment involving decreased localization of molecules important for cell–cell interactions in RL95-2 cells.

Keywords: BaP; TCDD; Attachment; Adhesion; EGF-R; Cadherin; β-catenin; Cytoskeleton; Human uterine endometrium

Evidence from epidemiological and laboratory studies supports a role for environmental toxicants in uterine endometrial disease. Recent studies in rhesus monkeys and rodents indicate that 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), a prototype organochlorine, and related compounds may promote endometriosis [1–3]. In addition, human epidemiological data provide some evidence for an association between dioxin exposure and the promotion of uterine disease [4,5]. TCDD, a classical, non-genotoxic carcinogen, binds to the aryl hydrocarbon receptor (AhR) and has recently been shown to induce the expression of cytochrome P450 1A1 (CYP1A1) and 1B1 (CYP1B1) in explant cultures of human endometrial tissue [6,7], as well as human endometrial cell lines [8,9].

A second major environmental exposure is cigarette smoke, and women who smoke are known to have an increased risk of infertility and adverse pregnancy outcomes [10]. An unexpected finding is that smokers also have less than half the risk of developing endometrial cancer, endometrial hyperplasia, uterine fibroids, and endometriosis [10–12]. Little is known, however, regarding cellular mechanisms that may underlie the seemingly protective effects of cigarette smoke on endometrial tissue. Benzo(*a*)pyrene (BaP) is a polyaromatic hydrocarbon compound that is a major toxicant in cigarette smoke. BaP is a potent genotoxin that binds to the AhR and transcriptionally activates CYP1A1,

☆ *Abbreviations:* AhR, aryl hydrocarbon receptor; BaP, benzo(*a*)pyrene; DMSO, dimethyl sulfoxide; EGF-R, epidermal growth factor receptor; FBS, fetal bovine serum; PBS, phosphate buffered saline; SDS, sodium dodecyl sulfate; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

* Corresponding author. Fax: +1-352-392-9696.

E-mail address: kshiveri@pharmacology.ufl.edu (K.T. Shiverick).

[1] Present address: The Dow Chemical Company, Toxicology & Environmental Research & Consulting, Midland, MI.

0006-291X/02/$ - see front matter
PII: S0006-291X(02)00437-0

which is a pathway for further metabolism of BaP to form reactive metabolites and oxygen radicals [13].

Exposure of RL95-2 human uterine endometrial cells to TCDD has been reported to increase the expression of mRNA for several genes involved in cell adhesion as well as CYPs 1A1 and 1B1 [8,9]. The present study was undertaken to further investigate the effects of the two AhR-ligands, TCDD and BaP, on the ability of RL95-2 cells to attach to Matrigel-coated membranes and on the expression of key adherens junction and cytoskeletal proteins. Cell adhesion mechanisms play a fundamental role in the determination of tissue architecture and the functions of cell assembly and connection to the internal cytoskeleton [14]. The RL95-2 cell line was selected for these studies because of its relevance as an in vitro model for endometrial cell biology and cancer [15,16]. In addition, RL95-2 cells have been shown to exhibit adhesiveness of its apical pole for the trophoblast, thereby also serving as a model for human uterine epithelium receptive for implantation [16]. The results of the present study indicate that BaP, but not TCDD, produces a loss of cell attachment involving dysregulation of the localization of molecules important for cell–cell interactions in RL95-2 cells.

Materials and methods

Materials. TCDD was obtained from Midwest Research Institute (Kansas City, MO) through the National Cancer Institute Chemical Carcinogen Reference Repository; BaP was purchased from the Sigma Chemical (St. Louis, MO). Cell culture media was purchased from GIBCO BRL/Life Technologies (Gaithersburg, MD), fetal bovine serum (FBS) from Hyclone (Logan, UT), and Matrigel from Collaborative Biomedical Products (Bedford, MA). Polyclonal sheep anti-human EGF receptor antiserum was from Upstate Biotechnology (Lake Placid, NY), polyclonal goat anti-human cadherin antibody from Santa Cruz Biotechnology (Santa Cruz, CA), and monoclonal mouse anti-human β-catenin antiserum from Transduction (Lexington, KY).

Cell cultures and chemical treatments. The RL95-2 cell line was obtained from ATCC and maintained as previously described [8]. Stock solutions of TCDD and BaP were prepared in DMSO and added to cultures in complete medium with a final DMSO concentration of 0.1%.

In vitro attachment assay. The Matrigel attachment assay was performed using a modified Boyden Chamber apparatus [17]. RL95-2 cultures were incubated with BaP and TCDD for 48 h, after which cells were trypsinized and collected by centrifugation. Cells were resuspended in Hank's buffer and washed twice, and cell numbers determined using a hemocytometer. Approximately (3×10^4 cells in 27 μl of serum-free media in the presence of the respective chemicals were aliquoted into the lower wells of the Boyden chamber. The wells were overlaid with a Matrigel-coated polyvinyl-pyrrolidone-free polycarbonate membrane (Nucleopore, 8 μm diameter pore size) and the apparatus was inverted and maintained at 37 °C/5% CO_2 for 2–10 h to allow for cell attachment. The cells attached to Matrigel were stained with Leukostat (Fisher Scientific, Lexington, MA) and quantitated by light microscopy. Cells were counted in quadruplicate wells for each treatment regimen.

Immunocytochemistry. Cells were plated on poly-L lysine-coated four-well chamber slides and treated for 48 h with 10 μM BaP, 10 nM TCDD, or DMSO vehicle. Cells were fixed in freshly prepared 3.7% paraformaldehyde in modified HBSS for 10 min at room temperature followed by PBS washes (10 mM KPO_4, 150 mM NaCl, pH 7.5). Cells were then blocked in 10% serum for 10 min at 37 °C followed by anti-human EGF-R antibody in PBS/1% BSA (10 μg/ml) overnight at 4 °C. In control experiments, the primary antibody was replaced with PBS alone. Slides were washed in PBS and endogenous peroxidase activity blocked by incubation with 0.3% H_2O_2 in PBS for 10 min. Cells were incubated with rabbit anti-sheep peroxidase-labeled secondary antibody (1:50 dilution; Southern Biotechnology Associates, Birmingham, AL) for 10 min followed by PBS washes. Diaminobenzidine tetrahydrochloride with $CoCl_2$ enhancement was used as substrate for visualization of EGF-R using a Zeiss Axiophot microscope. Cells were then counterstained with hematoxylin and slides mounted with Fluoromount G.

For phalloidin staining of actin filaments, cells were fixed and permeabilized, and then incubated with 25 μl of 1 μM fluorescein labeled phalloidin (Molecular Probes) in PBS/well. Cells were visualized by epifluorescence microscopy.

Western immunoblot analysis. Cell membrane fractions were prepared by washing cells in PBS, three freeze-thaw cycles, and centrifugation at 12,000*g* for 10 min to obtain a membrane pellet. Cell lysates were prepared in lysis buffer (50 mM Tris–HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150 mM NaCl; 1 mM EGTA; 1 mM PMSF; 1 μg/ml aprotinin, leupeptin, pepstatin; 1 mM Na_3VO_4;1 mM NaF), centrifuged at 12,000*g* for 15 min, and supernatants were retained for analysis. Protein samples (40 μg) were separated by 7.5% or 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) and were transferred overnight to nitrocellulose membranes according to the method of Towbin et al. [18]. Membranes were briefly stained with Ponceau Red to confirm uniform protein loading and transfer. For cadherin and β-catenin immunoblots, the membranes were blocked in 10% dried milk/TBS, washed, and incubated in respective primary antibody in 5% milk/TBS overnight at 4 °C (1:150 Cadherin, 1:500 β-catenin). Membranes were then incubated in appropriate horseradish peroxidase (HRP)-conjugated secondary antibodies (1:2000) in 5% milk/TBS for 2 h at room temperature with agitation, washed, and incubated in ECL peracid detection solution (Amersham Life Science). For EGF-R Western immunoblots, immunostaining was performed according to Wang et al. [19]. Membranes were incubated in sheep anti-human EGF receptor, followed by horseradish-peroxidase conjugated anti-sheep IgG.

Immunoreactive bands were quantitated by densitometry using Scion image software. For each experiment, negative controls were run using pre-absorption of primary antibody or omission of primary antibody to ensure specificity of cross-reactive bands.

Data analysis. All experiments were performed in triplicate. For analysis of scanned images, control lanes were standardized to 100% and treatments assessed relative to controls for each individual experiment using Student's *t* test.

Results

Cellular attachment on Matrigel

Cells were cultured in the presence of 10 μM BaP, 10 nM TCDD, or DMSO vehicle for 48 h. These concentrations of TCDD and BaP produced comparable induction of mRNA and protein for CYP1A1 (data not shown), as has been previously reported [8]. Cells were trypsinized and all treated cells exhibited greater than 95% viability as determined by tryphan blue dye exclusion. Pretreated cells were applied to Matrigel-coated

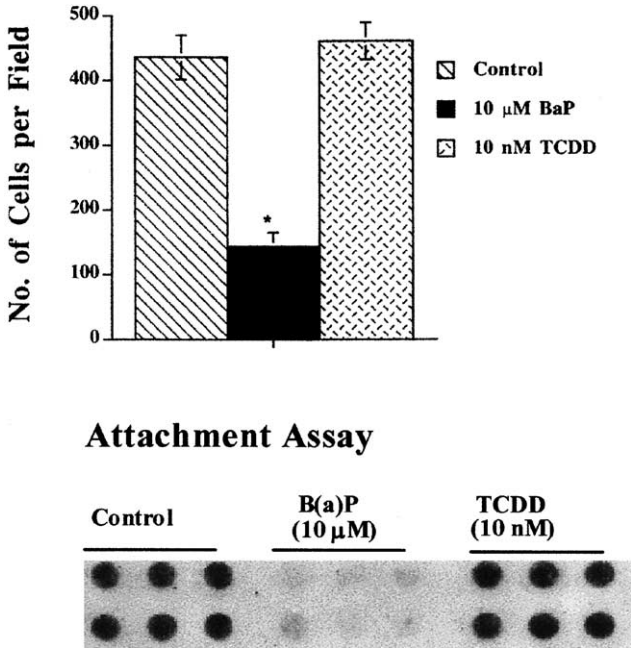

Fig. 1. Effects of BaP and TCDD on RL95-2 cell attachment to Matrigel-coated membranes. Cells were pre-treated for 48 h with 10 μM BaP, 10 nM TCDD, or 0.1% DMSO vehicle (control). Cells (3×10^4) in serum-free medium were aliquoted into the wells of a modified Boyden Chamber apparatus and allowed to attach to a Matrigel-coated membrane for 2 h. Attached cells were stained with Leukostat and counted by light microscopy. Upper panel, data are expressed as the means ± SEM from three separate experiments, $*p < 0.001$ compared to 0.1% DMSO. Lower panel, scanned image of treated RL95-2 cells attached to a Matrigel membrane.

membranes for 2 h, after which the membranes were stained for adherent nuclei. Fig. 1 shows that attachment of BaP-pretreated cells is significantly decreased by 70% compared to DMSO controls ($p < 0.001$), whereas attachment of TCDD-exposed cells was unaffected. This result was reproducible for attachment periods of up to 10 h. Thus, pretreatment with BaP, but not TCDD, markedly impaired the ability of RL95-2 cells to adhere to extracellular matrix constituents.

Effects on epidermal growth factor receptors (EGF-R)

Immunocytochemical analysis of cells grown on chamber slides showed that EGF-R immunoreactivity was primarily detected as intense brown immunostaining localized in intercellular regions of adjacent cells associated with the plasma membranes (Fig. 2A); a lesser amount of diffuse immunostaining was detected in the cytoplasm. Treatment with 10 μM BaP for 48 h resulted in a marked decrease in EGF-R staining along cell membranes, whereas cytoplasmic staining was still present (Fig. 2B). In contrast, 10 nM TCDD treatment produced no apparent effect in EGF-R localization relative to control (Fig. 2C). Replacement of the primary antibody with normal sheep serum or PBS showed a complete lack of EGF-R immunostaining (Fig. 2D).

Western immunoblot analysis confirmed the effect of BaP on cell membrane EGF-R (Fig. 2E). DMSO-treated control cells showed the expression of EGF-R as a 170 kDa protein in both cell membrane and cell lysate

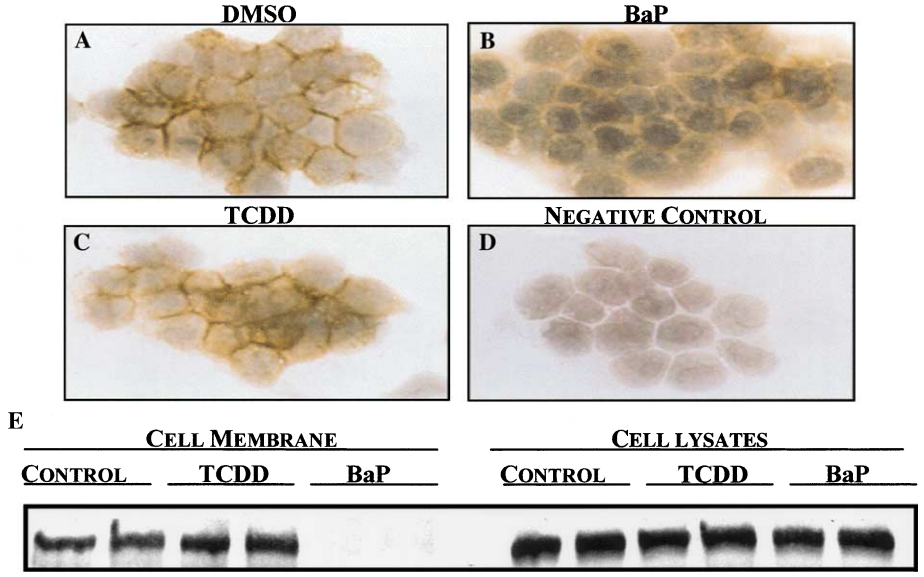

Fig. 2. Effects of BaP and TCDD on the localization and protein level of EGF-R in RL95-2 cells. A–D: Immunocytochemical localization of EGF-R in cells following 48 h treatment with DMSO vehicle control (A and D), 10 μM BaP (B), and 10 nM TCDD (C). Cells were incubated with sheep anti-human EGF-R antiserum (A–C) or PBS (D). Original magnification: 100×. E: Western immunoblot analysis of cell membrane preparations compared with whole cell lysates of cells following BaP and TCDD treatment. Samples (100 μg protein) were electrophoresed, transferred to nitrocellulose, and immunostained with anti-EGF-R antibody. Representative immunoblot of five separate experiments.

fractions. BaP treatment produced a total loss of plasma membrane EGF-R, while protein levels in whole cell lysates were not altered. This selective loss of membrane EGF-R with BaP was reproducibly found in over 10 experiments. In contrast, treatment with 10 nM TCDD had no effect on EGF-R expression levels. In the data not shown, 10 μM BaP caused a substantial loss of membrane EGF-R protein levels by 7 h treatment, an alteration that persists at 48 h.

Effects on cadherin and β-catenin cellular adhesion molecules

Studies next characterized xenobiotic effects on the cell adherens junction proteins, cadherin and β-catenin. Data in Fig. 3A show that cadherin is detected in RL95-2 cells as a 120 kDa protein. Treatment with 10 μM BaP, but not 10 nM TCDD, produced a significant $38 \pm 8\%$ decrease in cadherin levels in RL95-2 cell lysates ($p < 0.005$). β-Catenin, a second adherens junction protein, is detected as a 92 kDa protein in both cell membrane and cell lysate preparations of RL95-2 cells. Treatment with 10 μM BaP is associated with a significant 80% decrease in β-catenin protein level in cell membrane fractions (Fig. 3B), whereas cell lysate protein levels remained unchanged (data not shown). In contrast, treatment with TCDD had no effect on β-catenin levels in either preparation (Fig. 3B). In comparison, neither BaP nor TCDD treatments altered vinculin protein levels in cell membrane or lysate fractions (data not shown).

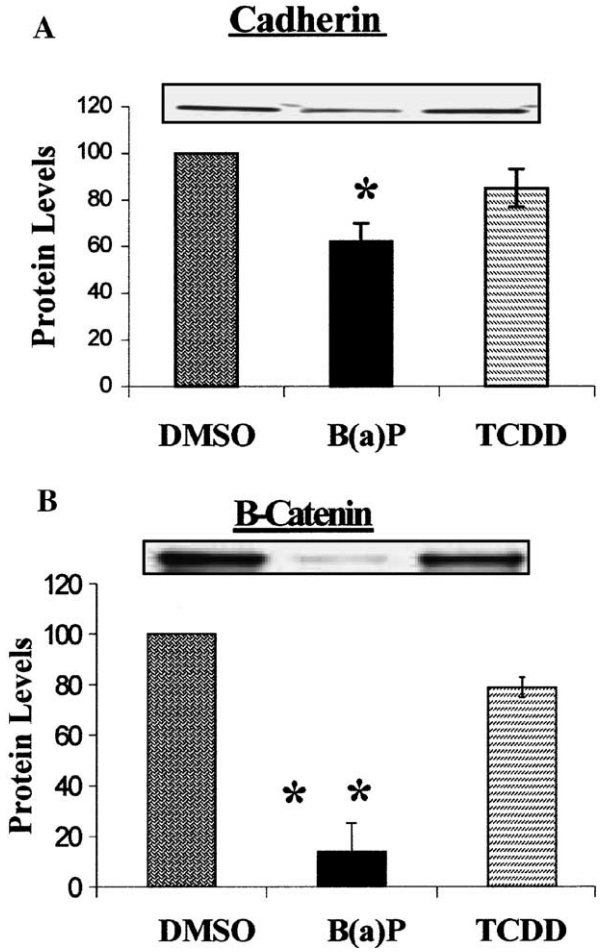

Fig. 3. Western immunoblot analysis of the effects of BaP and TCDD on (A) cadherin and (B) β-catenin levels. RL95-2 cells were treated with 10 μM BaP, 10 nM TCDD, or DMSO for 48 h and whole cell lysate (cadherin) or cell membrane (β-catenin) preparations were electrophoresed, transferred to nitrocellulose, and probed with the respective antibodies followed by horseradish peroxidase conjugated IgG. Data are expressed as the means ± SEM of three separate experiments. *$p < 0.005$ or **$p < 0.05$ compared to DMSO.

Effects on actin cytoskeletal protein

Subsequent immunocytochemical studies were performed investigating BaP-mediated effects on actin cytoskeletal localization in RL95-2 cells. Experiments utilized the fluorescein labeled peptide phalloidin to selectively visualize filamentous actin using fluorescence microscopy. Results indicate that filamentous actin is localized in a subcortical layer in control cells (Fig. 4A). In contrast, 10 μM BaP treatment is associated with the formation of subcortical actin aggregates in RL95-2 cells (Fig. 4B). However, despite the change in actin localization, Western immunoblot analysis of the 42 kDa actin protein in RL95-2 cells (Fig. 4C) showed that overall actin levels in cell membrane and triton X-100 fractions remain unaltered by BaP treatment.

Discussion

Uterine endometriosis is a benign proliferative disorder that affects millions of women, and endometrial cancer is the most common gynecologic malignancy in the United States. Recent studies provide evidence that environmental exposure to dioxins may promote endometriosis [1–5], while cigarette smoking is reported to decrease the incidence of endometriosis and uterine cancer [10–12]. Our laboratory is studying cultured human endometrial cells as an in vitro model to identify cellular and molecular biomarkers of exposure and effect following treatment with prototype organochlorines and constituents of cigarette smoke. In RL95-2 cells, TCDD has been previously reported to induce the expression of CYPs 1A1 and 1B1, as well as mRNA for genes involved in cell adhesion [8,9].

The present study demonstrates that major differential effects were observed on cell attachment in RL95-2 cells treated with the two AhR agonists BaP and TCDD. Our finding that TCDD had no effect on cell attachment in the presence of CYP1A1 induction does not support a direct relationship between the AhR and dysregulation

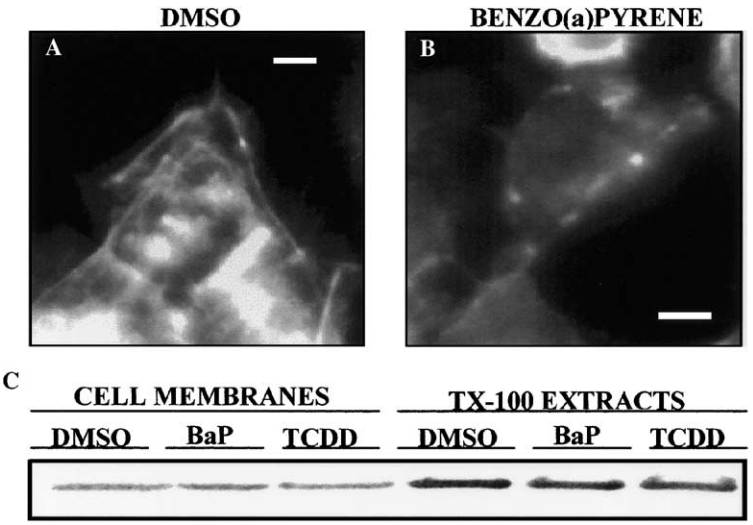

Fig. 4. Effects of BaP on the localization of actin filaments and protein levels in RL95-2 cells. A–B: Epi-fluorescent microscopy showing the immunocytochemical localization of actin filaments in RL95-2 cells following 48 h treatment with DMSO (A) and 10 μM BaP (B). Cells were incubated with fluorescein labeled phalloidin to visualize altered actin structure upon BaP treatment. The bar is 9 μm. C: Western immunoblot analysis of cell membrane and cell extract preparations following DMSO, BaP, and TCDD treatment. Samples containing 40 μg of protein were electrophoresed, transferred to nitrocellulose, and immunostained with anti-actin antibody. Representative immunoblot of three separate experiments.

of cell adhesion in RL95-2 cells. In this regard, recent studies of the effects of TCDD on explant cultures of human endometrium reported no endometriosis-related differences in the constitutive or TCDD-induced expression of AhR, ARNT, CYP1A1, or CYP1B1 biomarkers [6,7]. Since TCDD did not significantly alter cell attachment activity of the exposed explants, this agent may not act to promote endometriosis by enhancing implantation, but may perhaps act at a later phase of growth and proliferation or through a different mechanism [1,8].

In contrast, the present study found that BaP induced a significant decrease in cell attachment activity, which was associated with decreased localization of EGFR protein along cell membranes without a significant change in total immunoreactive EGFR protein. In this regard, cigarette smoking has been consistently associated with alterations in placental EGF receptors [19,20], an effect that is also produced by BaP treatment of cultured placental cells [21,22]. Activation of EGFR has been shown to directly regulate cell adhesion through effects on E-cadherin interactions with actin in a human breast cancer cell line [23]. The present study observed reorganization of cortical actin filament conformation into aggregates with BaP, but not TCDD treatment, reflecting another fundamental structural alteration following BaP treatment in RL95-2 cells. In this regard, actin bundles are recognized to attach to integral membrane cadherin and integrin proteins, adapter proteins and the contractile bundle [14]. Thus, actin works in conjunction with cytoskeletal microtubules and intermediate filaments in performing essential functions in locomotion and cytokinesis [14]. In the present study, the BaP-mediated loss of cell attachment in RL95-2 cells appears to be related to a rearrangement of cortical actin cytoskeletal structure.

The BaP-mediated alterations observed in RL95-2 cell attachment are further associated with alterations in expression of the adherens junction proteins, cadherin and β-catenin. Cadherins are integral membrane glycoproteins functioning in epithelial cells to form calcium-dependent linkages between cells [24,25]. Cadherins play a key role in mediating the formation and breakage of cell-to-cell contacts and in maintaining strength in cellular adhesion through homophilic interaction of their extracellular domains, allowing for cellular aggregation to occur [24]. Cadherins connect to β-catenin, a key membrane-associated protein responsible for the colocalization of cadherins to sites of cell–cell contact with the actin cytoskeleton [24]. In the present study, BaP-mediated decreases in the cadherin and β-catenin adherens junction proteins provide further evidence for the likely role of BaP in decreasing cellular attachment in RL95-2 cells.

A number of studies have reported that oxidative stress is a common mechanism for disruption of cell adhesion. Endothelial cells exposed to oxidative stress were found to have cadherin internalization, impairment in the expression and internalization of adherens junctional proteins, and actin reorganization associated with loss of the cortical actin filament band [26]. Oxidative stress in ocular cells produced loss of adhesion to extracellular matrix that was linked to reorganization of actin cytoskeletal structure, without changes in integrin expression [27]. Non-lethal oxidative stress in mouse hepatocytes was found to selectively disrupt the

E-cadherin/β-catenin complex, without altering other adherens junction proteins [28,29]. Seagrave and Burchiel [30] found that treatment of MCF-10A non-transformed human mammary epithelial cells with BaP and UV light resulted in the reorganization of actin filaments into substrate-associated aggregates. These effects were associated with depletion of cellular ATP and proposed to occur through UVA-mediated alterations in BaP metabolism and generation of oxidative stress. In the present study, metabolism of BaP by induced CYP1A1 and CYP1B1 is a likely mechanism for production of oxidative damage in RL95-2 endometrial cells, which would further account for the lack of effect of TCDD since it does not undergo metabolic activation by these enzymes [31,32].

Finally, the observed adverse effect of BaP on attachment in RL95-2 cells may be a phenotype consistent with the decreased incidence of endometriosis, as well as infertility found in cigarette smokers [10–12]. In this regard, the RL95-2 cell line is distinct from other human endometrial cell lines in that it exhibits an adhesiveness of its apical pole for trophoblast and thereby serves as an in vitro model for the human uterine epithelium receptive for implantation [16]. The BaP-mediated dysregulation of cell adhesion molecules in RL95-2 cells may serve as a useful biomarker for alterations in uterine epithelial cell polarization and subsequent effects on altered trophoblast implantation. Thus the phenotype associated with BaP treatment would be potentially beneficial to suppress benign adhesive uterine disorders, while proving unfavorable for implantation and the establishment of pregnancy.

Acknowledgments

This publication was supported by Grant ES 07375 from the Superfund Basic Research Program (KTS), National Institute of Environmental Health Sciences with funds from EPA. Its contents are solely the responsibility of the authors and do not necessarily reflect the official views of the NIEHS, NIH, or EPA.

References

[1] L.S. Birnbaum, A.M. Cummings, Dioxins and endometriosis: a plausible hypothesis, Environ. Health Perspect. 110 (2002) 15–21.

[2] S.E. Rier, D.C. Martin, R.E. Bowman, W.P. Dmowski, J.L. Becker, Endometriosis in rhesus monkeys (Macaca mulatta) following chronic exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, Fundam. Appl. Toxicol. 21 (1993) 433–441.

[3] A.M. Cummings, J.L. Metcalf, L. Birnbaum, Promotion of endometriosis by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in rats and mice: time-dose dependence and species comparison, Toxicol. Appl. Pharmacol. 138 (1996) 131–139.

[4] P.R. Koninckx, P. Braet, S.H. Kennedy, D.H. Barlow, Dioxin pollution and endometriosis in Belgium, Hum. Reprod. 9 (1994) 1001–1002.

[5] A. Mayani, S. Barel, S. Soback, M. Almagor, Dioxin concentrations in women with endometriosis, Hum. Reprod. 12 (1997) 373–375.

[6] J.A. Pitt, L. Feng, B.A. Abbott, J. Schmid, R.E. Batt, T.G. Costich, S.T. Koury, D.P. Bofinger, Expression of AhR and ARNT mRNA in cultured human endometrial explants exposed to TCDD, Toxicol. Sci. 62 (2001) 289–298.

[7] D.P. Bofinger, L. Feng, L.-H. Chi, J. Love, F.D. Stephen, T.R. Sutter, K.G. Osteen, T.G. Costich, R.E. Batt, S.T. Loury, J.R. Olson, Effect of TCDD exposure on CYP1A1 and CYP1B1 in explant cultures of human endometrium, Toxicol. Sci. 62 (2001) 299–314.

[8] G.D. Charles, K.T. Shiverick, 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin increases mRNA levels for interleukin-1beta, urokinase plasminogen activator, and tumor necrosis factor-alpha in human uterine endometrial adenocarcinoma RL95-2 cells, Biochem. Biophys. Res. Commun. 238 (1997) 338–342.

[9] N.R. Jana, S. Sarkar, M. Ishizuka, J. Yonemoto, C. Tohyama, H. Sone, Comparative effects of TCDD on MCF-7, RL95-2 and LNCaP cells: role of target steroid hormones in cellular responsiveness to CYP1A1 induction, Mol. Cell. Biol. Res. Commun. 4 (2000) 174–180.

[10] K.T. Shiverick, C. Salafia, Cigarette smoking and pregnancy I: ovarian, uterine and placental effects, Placenta 20 (1999) 265–272.

[11] D.W. Cramer, E. Wilson, R.J. Stillman, M.J. Berger, S. Belisle, I. Schiff, B. Albrecht, M. Gibson, B.V. Stadel, S.C. Schoenbaum, The relation of endometriosis to menstrual characteristics, smoking, and exercise, J. Am. Med. Assoc. 255 (1986) 1904–1908.

[12] J.A. Baron, C. La Vecchia, F. Levi, The antiestrogenic effect of cigarette smoking in women, Am. J. Obstet. Gynecol. 162 (1990) 502–514.

[13] J.P. Whitlock Jr., S.T. Okino, L. Dong, H.P. Ko, R. Clarke-Katzenberg, Q. Ma, H. Li, Cytochromes P450 5: induction of cytochrome P4501A1: a model for analyzing mammalian gene transcription, FASEB J. 10 (1996) 809–818.

[14] M.P. Quinlan, J.L. Hyatt, Establishment of the circumferential actin filament network is a prerequisite for localization of the cadherin–catenin complex in epithelial cells, Cell Growth Differ. 10 (1999) 839–854.

[15] D.L. Way, D.S. Grosso, J.R. Davis, E.A. Surwit, C.D. Christian, Characterization of a new human endometrial carcinoma (RL95-2) established in tissue culture, In Vitro 19 (1983) 147–158.

[16] M. Thie, P. Herter, H. Pommerenke, F. Durr, F. Sieckmann, B. Nebe, J. Rychly, H.W. Denker, Adhesiveness of the free surface of a human endometrial monolayer for trophoblast as related to actin cytoskeleton, Mol. Hum. Reprod. 3 (1997) 275–283.

[17] M. Grant, J. Jerdan, T.J. Merimee, Insulin-like growth factor-I modulates endothelial cell chemotaxis, J. Clin. Endocrinol. Metab. 65 (1987) 370–371.

[18] H. Towbin, T. Staehelin, J. Gordon, Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications, Proc. Natl. Acad. Sci. USA 76 (1979) 4350–4354.

[19] S.L. Wang, G.W. Lucier, R.B. Everson, G.I. Sunahara, K.T. Shiverick, Smoking-related alterations in epidermal growth factor and insulin receptors in human placenta, Mol. Pharmacol. 34 (1988) 265–271.

[20] G.I. Sunahara, K.G. Nelson, T.K. Wong, G.W. Lucier, Decreased human birth weights after *in utero* exposure to PCBs and PCDFs are associated with decreased placental EGF-stimulated receptor autophosphorylation capacity, Mol. Pharmacol. 32 (1987) 572–578.

[21] L. Zhang, E.E. Conner, N. Chegini, K.T. Shiverick, Modulation by benzoapyrene of epidermal growth factor receptors, cell proliferation and secretion of human chorionic gonadotropin in human placental cell lines, Biochem. Pharmacol. 50 (1995) 1171–1180.

[22] L. Zhang, K.T. Shiverick, Benzo(*a*)pyrene, but not TCDD, alters cell proliferation and c-myc and growth factor expression in human placental choriocarcinoma JEG-3 cells, Biochem. Biophys. Res. Commun. 231 (1997) 117–120.
[23] R.B. Hazan, L. Norton, The epidermal growth factor receptor modulates the interaction of E-cadherin with the actin cytoskeleton, J. Biol. Chem. 273 (1998) 9078–9084.
[24] E. Potter, C. Bergwitz, G. Brabant, The cadherin–catenin system: implications for growth and differentiation of endocrine tissues, Endocr. Rev. 20 (1999) 207–239.
[25] K.A. Knudsen, A.P. Soler, Cadherin-mediated cell–cell interactions, Methods Mol. Biol. 137 (2000) 409–440.
[26] H. Lum, K.A. Roebuck, Oxidant stress and endothelial cell dysfunction, Am. J. Physiol. Cell Physiol. 280 (2001) C719–C741.
[27] L. Zhou, Y. Li, B.Y. Yue, Oxidative stress affects cytoskeletal structure and cell–matrix interactions in cells from an ocular tissue: the trabecular meshwork, J. Cell. Physiol. 180 (1999) 182–189.
[28] A.R. Parrish, J.M. Catania, J. Orozco, A.J. Gandolfi, Chemically induced oxidative stress disrupts the E-cadherin/catenin cell adhesion complex, Toxicol. Sci. 51 (1999) 80–86.
[29] M. Schmelz, V.J. Schmid, A.R. Parrish, Selective disruption of cadherin/catenin complexes by oxidative stress in precision-cut mouse liver slices, Toxicol. Sci. 61 (2001) 389–394.
[30] J.C. Seagrave, S.W. Burchiel, Interactions between benzo[a]pyrene and UVA light affecting ATP levels, cytoskeletal organization, and resistance to trypsinization, Toxicol. Lett. 117 (2000) 11–23.
[31] M.C. Larsen, W.G. Angus, P.B. Brake, S.E. Eltom, K.A. Sukow, C.R. Jefcoate, Characterization of CYP1B1 and CYP1A1 expression in human mammary epithelial cells: role of the aryl hydrocarbon receptor in polycyclic aromatic hydrocarbon metabolism, Cancer Res. 58 (1998) 2366–2374.
[32] S.A. Leadon, M.R. Stampfer, J. Bartley, Production of oxidative DNA damage during the metabolic activation of benzo(*a*)pyrene in human mammary epithelial cells correlates with cell killing, Proc. Natl. Acad. Sci. USA 85 (1988) 4365–4368.