Vitamin D Receptor Activation Enhances Benzo[a]pyrene Metabolism via CYP1A1 Expression in Macrophages

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ABSTRACT:

Benzo[a]pyrene (BaP) activates the aryl hydrocarbon (AHR) and induces the expression of genes involved in xenobiotic metabolism, including CYP1A1. CYP1A1 is involved not only in BaP detoxification but also in metabolic activation, which results in DNA adduct formation. Vitamin D receptor (VDR) belongs to the NR1I subfamily of the nuclear receptor superfamily, which also regulates expression of xenobiotic metabolism genes. We investigated the cross-talk between AHR and VDR signaling pathways and found that 1α,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃], a potent physiological VDR agonist, enhanced BaP-induced transcription of CYP1A1 in human monocytic U937 cells and THP-1 cells, breast cancer cells, and kidney epithelium-derived cells. 1,25(OH)₂D₃ alone did not induce CYP1A1, and 1,25(OH)₂D₃ plus BaP did not increase CYP1A2 or CYP1B1 mRNA expression in U937 cells. The combination of 1,25(OH)₂D₃ and BaP increased CYP1A1 protein levels, BaP hydroxylation activity, and BaP-DNA adduct formation in U937 cells and THP-1 cells more effectively than BaP alone. The combined effect of 1,25(OH)₂D₃ and BaP on CYP1A1 mRNA expression in U937 cells and/or THP-1 cells was inhibited by VDR knockdown, VDR antagonists, and α-naphthoflavone, an AHR antagonist. Electrophoretic mobility shift assays and chromatin immunoprecipitation assays showed that VDR directly bound to an everted repeat (ER) 8 motif in the human CYP1A1 promoter. Thus, CYP1A1 is a novel VDR target gene involved in xenobiotic metabolism. Induction of CYP1A1 by the activation of VDR and AHR may contribute to BaP-mediated toxicity and the physiological function of this enzyme.

Introduction

Polycyclic aromatic hydrocarbons such as benzo[a]pyrene (BaP) are environmental pollutants produced by the combustion of cigarettes, creosote railroad ties, and coke ovens (Miller and Ramos, 2001; Uno and Makishima, 2009). BaP is implicated as a causative agent in malignancies, such as lung and head-and-neck cancers, and atherosclerosis as a consequence of cigarette smoking (Shimada and Fujii-Kuriyama, 2004; Alexandrov et al., 2010). BaP inhalation activates the aryl hydrocarbon (AHR) receptor, which forms an active transcription factor heterodimer with the AHR nuclear translocator, and induces expression of a group of genes called the [Ah] gene battery, which includes the phase I enzymes [cytochrome P450 (P450) 1A1 (gene symbol, CYP1A1), CYP1A2, CYP1B1, and NAD(P)H:quinone oxidoreductase 1] and the phase II enzymes (glutathione transferase A1 and UDP glucuronosyltransferase 1A6) (Nebert et al., 2000).

BaP mediates carcinogenic, mutagenic, and cytotoxic effects after conversion to toxic metabolites through an AHR-dependent mechanism of metabolic activation (Miller and Ramos, 2001; Shimada and Fujii-Kuriyama, 2004). BaP is first oxidized by CYP1A1 and CYP1B1 to phenols, such as 3-hydroxy-BaP and 9-hydroxy-BaP, and epoxides, such as BaP-7,8-epoxide (Shimada, 2006; Uno and Makishima, 2009). BaP-7,8-epoxide is then metabolized by epoxide hydrolase to BaP-7,8-diol, which serves as substrate for a second P450-dependent oxidation, generating the toxic compound BaP-7,8-diol-9,10-epoxide. Among the metabolites in BaP-treated cells, (+)-BaP-7,8-diol-9,10-epoxide-2 is the most reactive carcinogen (Shimada, 2006; Alexandrov et al., 2010).

Original studies on mutant Hepa-1 cells that are resistant to BaP-induced growth suppression have shown that BaP resistance is associated...
cated with mutations in the Cyp1a1 gene and dysfunction of the AHR transcription factor (Hankinson et al., 1991). Expression of exogenous CYP1A1 in CYP1A1-deficient cells restores the formation of BaP-induced DNA adducts (Maier et al., 2002). These findings indicate that metabolic activation of BaP requires the AHR-CYP1A1 cascade. In contrast, BaP-induced DNA adducts are increased in the liver, and BaP clearance from the blood is slower in CYP1A1-null mice (Uno et al., 2001). Overexpression of CYP1A1 in hepatocytes suppresses BaP-induced DNA adduct formation and AHR transactivation (Endo et al., 2008). CYP1A1 may be involved in both metabolic activation and detoxification of BaP depending on conditions (Uno et al., 2006).

The active form of vitamin D₃, 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃], binds to the vitamin D receptor (VDR; NR1I1) and regulates numerous physiological and pharmacological processes, including bone and calcium metabolism, cellular growth and differentiation, immunity, and cardiovascular function (Nagpal et al., 2005; Choi and Makishima, 2009). Natural and synthetic VDR ligands inhibit the proliferation and/or induce the differentiation of various types of malignant cells, including myeloid leukemia (Hozumi, 1998; Nagpal et al., 2005). On ligand binding, VDR undergoes conformational changes that result in dynamic interaction with the heterodimer partner retinoid X receptor (RXR; NR2B) and exchange of cofactor complexes (Makishima and Yamada, 2005). Corepressors bind to the VDR-RXR heterodimer in the absence of ligand, and ligand binding reduces the affinity of corepressors and increases the affinity for coactivators, a structural transition that induces transcription of specific genes. The VDR-RXR heterodimer binds preferentially to a vitamin D response element that consists of a two-hexanucleotide (AGGTCA or a related sequence) direct repeat motif separated by three nucleotides. An inverted palindromic of the hexanucleotide motif, also called reverted repeat (ER) element (Mangelsdorf and Evans, 1995), separated by six, seven, eight, or nine nucleotides has also been identified as vitamin D response elements in genes including CYP3A4 (Thummel et al., 2001; Choi and Makishima, 2009). VDR has been found to act as a receptor for secondary bile acids, including lithocholic acid (LCA) and 3-ketocholanic acid, and to induce the expression of CYP3A enzymes (Makishima et al., 2002). CYP3A enzymes catalyze the metabolic conversion of a wide variety of xenobiotics and endogenous substrates, including bile acids, to more polar derivatives (Xie and Evans, 2001).

VDR belongs to the NR1I nuclear receptor subfamily along with pregnane X receptor (NR1I2) and constitutive androstane receptor (CAR; NR1I3), both of which play a role in the regulation of xenobiotic metabolism. These findings suggest that VDR may be involved in the regulation of xenobiotic-metabolizing enzymes other than CYP3A. Recently, CAR has been found to induce the expression of CYP1A1 and CYP1A2 expression by binding to a common regulatory element in the human CYP1A1 and CYP1A2 genes in hepatocytes (Yoshinari et al., 2010). In this study, we report that VDR activation enhances AHR-induced CYP1A1 expression in human macrophage-derived cells (American Type Culture Collection, Manassas, VA) were cultured in RPMI 1640 medium containing 10% fetal bovine serum, 100 units/ml penicillin, and 100 µg/ml streptomycin at 37°C in a humidified atmosphere containing 5% CO₂. Human kidney HEK293 cells (RIKEN Cell Bank) were cultured in Dulbecco’s modified Eagle medium containing 5% fetal bovine serum. Cell viability after all of the treatments was more than 90%, as determined by exclusion of trypan blue.

Reverse Transcription and Real-Time Quantitative Polymerase Chain Reaction. Total RNAs from samples were prepared by the acid guanidinium thiocyanate-phenol/chloroform method (Tavangar et al., 1990; Matsunawa et al., 2009). cDNAs were synthesized using the ImProm-II Reverse Transcription system (Promega Corporation, Madison, WI). Real-time polymerase chain reaction (PCR) was performed on the ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Foster City, CA) using Power SYBR Green PCR Master Mix (Applied Biosystems). Intron-spanning primers were as follows: CYP1A2 (GenBank accession number NM_000761), 5′-AGT GGG AGA TCT TCT TCT TC-3′ and 5′-GGT GTC TTC TTC AGT TGA TG-3′; VDR (GenBank accession number NM_000376), 5′-CAC GCT ACT GAC GGC GGT GAT TT-3′ and 5′-GCT GAC CTC GTC AGT TAC ACC A-3′; AHR (GenBank accession number NM_001621), 5′-GTA GTC CCT TCA TAC C-3′ and 5′-AGG CAC GAA TTG GTT AGA G-3′. Other primers were prepared previously (Matsunawa et al., 2009). The RNA values were normalized to the amount of β-actin mRNA, or mRNA copy numbers were determined using expression plasmids for a standard curve in real-time PCRs (Uno et al., 2006).

Western Blot Analysis. For VDR and AHR expression, nuclear extracts were prepared as described previously (Schreiber et al., 1989; Inaba et al., 2007). For CYP1A1 expression, microsomes (59 fraction) from cells were prepared as described previously (Uno et al., 2001; Endo et al., 2008). Western blot analysis was performed using a monoclonal anti-VDR antibody (Santa Cruz Biotechnology, Santa Cruz, CA), a polyclonal anti-AHR antibody (R&D Systems Inc., Minneapolis, MN), a monoclonal anti-CYP1A1 antibody (Daichi Pure Chemicals, Tokyo, Japan), and a monodonal anti-β-actin antibody (Sigma-Aldrich), visualized with an enhanced chemiluminescence detection system or an alkaline phosphatase conjugate substrate system (Endo et al., 2008).

Enzyme Activity Assays. Extracts from microsomal fractions were subjected for enzyme activity assays. BaP hydroxylation was assayed as previously reported (Nebert and Gelboin, 1968; Endo et al., 2008). In brief, 200 µg of microsomal protein was incubated in 100 mM potassium phosphate buffer (pH 7.4) containing 1 mg/ml bovine serum albumin, 80 µM BaP, and 0.5 mM NaN₃ at 37°C for 20 min. The reaction was stopped by the addition of acetonitrile-hexane (1:3). After the organic phase was extracted with NaOH, the concentration of 3-hydroxy-BaP was measured spectrofluorometrically with an enhanced chemiluminescence detection system or a standard curve in real-time PCRs (Uno et al., 2006).

Measurement of DNA Adducts. BaP-induced DNA adducts were determined by a ³²P-postlabeling method (Talaska et al., 1996; Uno et al., 2001; Endo et al., 2008). After DNA extraction from cells, hydrolysis to 3′-phosphodideoxyribonucleotides with spleen phosphodiesterase, and removal of nonadenucleotides with β-nicotinamide adenine dinucleotide, the 3′-phosphodideoxyribonucleotides were labeled at the 5′ positions with ³²P-ATP and T4 polynucleotide kinase. Two-dimensional thin-layer chromatography on polyethyleneimine cellulose sheets was used to resolve the ³²P-labeled DNA adducts (Randerath and Randerath, 1964), which were then visualized and quantified by scintillation counting (Packard 1900 CA; PerkinElmer Life and Analytical Sciences, Waltham, MA). Specific activity of isotope-labeled ATP was expressed as relative adduct labeling values normalized with amounts of DNA in each sample.

RNA Interference. Small interfering RNAs (siRNAs) directed against VDR (Dharmacon siGENOME SMARTpool) and control siRNA (Dharmacon siGENOME nontargeting siRNA pool) were purchased from Thermo Fisher Scientific (Waltham, MA). siRNA oligonucleotides were transfected into cells using DharmaFECT1 Reagent (Thermo Fisher Scientific) according to the manufacturer’s instructions.

Electrophoretic Mobility Shift Assays. Electrophoretic mobility shift assays (EMSAs) were performed as reported previously (Yoshikawa et al., 2001; Endo-Umeda et al., 2012). In brief, receptor proteins were in vitro translated with a TNT Quick Coupled Transcription/Translation System (Promega Corporation). Sequences for double-stranded oligonucleotides are shown in Fig. 2060 MATSUNAWA ET AL.
A. Binding reactions were performed in a buffer containing 10 mM Tris-HCl (pH 7.6), 50 mM KCl, 0.05 mM EDTA, 2.5 mM MgCl₂, 8.5% glycerol, 1 mM dithiothreitol, 0.5 μg/ml poly(dI-dC), 0.1% Triton X-100, and nonfat milk. Unlabeled probes and anti-VDR antibody (Santa Cruz Biotechnology) were used for competition experiments and supershift experiments, respectively. Samples were separated on 5% polyacrylamide gels and were visualized with autoradiography.

Chromatin Immunoprecipitation. Chromatin immunoprecipitation (ChIP) was performed as reported previously (Shang et al., 2000; Matsunawa et al., 2009). After nuclear proteins were cross-linked to DNA in 1% formaldehyde for 15 min, cells were washed and lysed in lysis buffer (50 mM Tris-HCl, pH 8.1, 1% SDS, and 10 mM EDTA). After sonication and removal of cellular debris, the lysates were diluted in ChIP dilution buffer (16.7 mM Tris-HCl, pH 8.1, 0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, and 167 mM NaCl). ChIP was performed with control IgG antibody, anti-AHR antibody, or anti-VDR antibody (Santa Cruz Biotechnology). DNA was purified with MonoFas DNA Purification Kit (GL Sciences, Torrance, CA). PCR was performed using GoTaq Master Mix (Promega) with the following primers: 5'-GAA CGC TGG GCG TGC AGA TGC CTC-3' and 5'-CAC TAA GGC GAT CCT AGA GGC TG-3', detecting the region 375 to 693 in CYP1A1 promoter as shown in Fig. 5A. The PCR products were separated by electrophoresis in 2% agarose gel.

Statistical Analyses. All values are shown as means ± S.E.M. The two-tailed, unpaired Student’s t test was performed to assess significant differences.

Results

1,25(OH)₂D₃ Enhances the Expression of CYP1A1 Induced by BaP. We previously reported that AHR activation by BaP and TCDD enhances the expression of CYP24A1 in human monocyte/macrophage-derived THP-1 cells treated with 1,25(OH)₂D₃ (Matsunawa et al., 2009). To further investigate the reciprocal effects of VDR and AHR signaling, we treated monocyte-derived U937 cells and THP-1 cells with BaP and/or 1,25(OH)₂D₃ for 24 h. The values represent means ± S.E.M. of triplicate assays. *p < 0.05; **p < 0.01.

5A. Binding reactions were performed in a buffer containing 10 mM Tris-HCl (pH 7.6), 50 mM KCl, 0.05 mM EDTA, 2.5 mM MgCl₂, 8.5% glycerol, 1 mM dithiothreitol, 0.5 μg/ml poly(dI-dC), 0.1% Triton X-100, and nonfat milk. Unlabeled probes and anti-VDR antibody (Santa Cruz Biotechnology) were used for competition experiments and supershift experiments, respectively. Samples were separated on 5% polyacrylamide gels and were visualized with autoradiography.

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Statistical Analyses. All values are shown as means ± S.E.M. The two-tailed, unpaired Student’s t test was performed to assess significant differences.
CYP1A1, an AHR target gene that has been shown to be involved in metabolic activation and detoxification of BaP (Uno and Makishima, 2009). Treatment with BaP (0.3 μM) induced the expression of CYP1A1 in U937 cells and THP-1 cells, whereas 1,25(OH)D3 (30 nM) did not show significant induction (Fig. 1A). CYP1A1 mRNA expression induced by BaP was further increased by coadministration of 1,25(OH)D3 in these cells (Fig. 1A). Combined BaP and 1,25(OH)D3 treatment also increased CYP1A1 mRNA expression to a higher degree than by BaP alone in breast cancer MCF-7 cells and in kidney epithelium-derived HEK293 cells (Fig. 1A). Previous reports have demonstrated expression of functional VDR and AHR proteins in U937 cells, THP-1 cells, MCF-7 cells, and HEK293 cells (Hayashi et al., 1995; Campbell et al., 2000; Inaba et al., 2007; Ishizawa et al., 2008; Zhang et al., 2008; Amano et al., 2009). We compared mRNA and protein levels of VDR and AHR in these cell lines. VDR mRNA expression levels in U937 cells, THP-1 cells, MCF-7 cells, and HEK293 cells were 1008 ± 126 copies, 51 ± 9 copies, 690 ± 88 copies, and 526 ± 28 copies/μg total RNA, respectively, whereas those for AHR were 856 ± 244 copies, 107 ± 19 copies, 7232 ± 922 copies, and 1900 ± 217 copies/μg total RNA, respectively (Fig. 1B). We next examined expression of nuclear VDR and AHR protein levels. As reported previously (Amano et al., 2009), VDR protein expression was observed in U937 cells and, to a lesser extent, in THP-1 cells in the absence of ligand (Fig. 1C). BaP plus 1,25(OH)D3 increased VDR protein levels in these cells as well as in MCF-7 cells and HEK293 cells (Fig. 1C). AHR protein expression was observed in all cell lines both with and without combined BaP and 1,25(OH)D3 treatment (Fig. 1C). There were some discrepancies between mRNA and protein levels of VDR and AHR (Fig. 1, B and C). It may be due to translational or posttranslational regulation of these proteins. As reported previously (Ishizawa et al., 2008; Matsunawa et al., 2009), 1,25(OH)2D3 treatment effectively induced expression of the VDR target CYP24A1 in U937 cells and THP-1 cells (Fig. 1D), indicating that VDR functions in these cells. BaP (1 μM) did not induce CYP1A2 or CYP1B1 expression in U937 cells, and the combination of BaP and 1,25(OH)2D3 had no effect on expression of these genes (Fig. 1E). These findings indicate that combined BaP and 1,25(OH)2D3 treatment effectively induces CYP1A1 mRNA expression.

Next, we examined the effects of several concentrations of BaP in combination with 1,25(OH)2D3 on CYP1A1 mRNA expression in U937 cells. In the absence of 1,25(OH)2D3, BaP at 0.1 μM increased CYP1A1 mRNA expression, an effect not seen at 0.01 or 0.03 μM (Fig. 2A). In combination with 10 nM 1,25(OH)2D3, BaP at 0.01 μM effectively induced CYP1A1 expression. Although BaP alone exhibited maximal CYP1A1 induction at 0.1 μM, the combination of 1,25(OH)2D3 further enhanced CYP1A1 expression in U937 cells treated with 0.1, 0.3, and 1 μM BaP (Fig. 2A). 1,25(OH)2D3 alone up to 100 nM did not increase CYP1A1 expression. 1,25(OH)2D3 at 3 to 30 nM increased CYP1A1 expression induced by 0.3 μM BaP (Fig. 2B). Thus, enhanced BaP-induced CYP1A1 expression by 1,25(OH)2D3 is concentration-dependent.

The effects of combined BaP and 1,25(OH)2D3 on CYP1A1 protein levels and enzyme activity were examined. BaP treatment induced CYP1A1 protein expression in U937 cells, and the addition of 1,25(OH)2D3 further increased CYP1A1 protein level, whereas 1,25(OH)2D3 alone caused modest induction (Fig. 3A). Although CYP1A1 proteins were detected only at low levels in THP-1 cells treated with BaP or 1,25(OH)2D3 alone, combined BaP and 1,25(OH)2D3 treatment effectively induced CYP1A1 protein expression in THP-1 cells (Fig. 3A). We examined BaP hydroxylation activity by detecting conversion of BaP to 3-hydroxy-BaP. CYP1A1, but not CYP1A2 or CYP1B1, mediate this cellular reaction (Endo et al., 2008). Cells were treated with BaP and/or 1,25(OH)2D3 for 24 h, and microsomes were isolated for enzyme activity assays. BaP treatment effectively increased BaP hydroxylation activity in U937 cells (Fig. 3B). 1,25(OH)2D3 slightly increased BaP hydroxylation activity in U937 cells (Fig. 3B), consistent with CYP1A1 protein expression (Fig. 3A) but not with mRNA expression (Figs. 1A and 2). 1,25(OH)2D3 may increase CYP1A1 protein levels and enzymatic activity through an unknown posttranslational mechanism. Combined BaP and 1,25(OH)2D3 treatment further increased BaP hydroxylation activity in U937 cells (Fig. 3B). BaP treatment also increased BaP hydroxylation activity in THP-1 cells, but the enzyme activity levels...
were very weak compared with U937 cells (Fig. 3B). This difference is likely due to lower CYP1A1 protein levels in THP-1 cells (Fig. 3A). Although 1,25(OH)₂D₃ alone was not effective, it enhanced BaP hydroxylation activity in THP-1 cells treated with BaP (Fig. 3B). Exogenous CYP1A1 expression increases and decreases BaP-DNA adduct formation in Hepa-1 cells and HepG2 cells, respectively (Maier et al., 2002; Endo et al., 2008). We next examined the effect of 1,25(OH)₂D₃ on BaP-DNA adduct formation. BaP-DNA adduct formation was detected in BaP-treated U937 cells and THP-1 cells, and combined 1,25(OH)₂D₃ treatment further increased BaP-DNA adducts in these cells (Fig. 3C). Therefore, BaP and 1,25(OH)₂D₃ cotreatment increases CYP1A1 protein levels, BaP hydroxylation activity, and BaP-DNA adduct formation in U937 cells and THP-1 cells.

Both VDR Activation and AHR Activation Are Involved in CYP1A1 Transcription. VDR is activated by LCA and its derivatives, such as LCA acetate, as well as 1,25(OH)₂D₃ (Ishizawa et al., 2008). Both ADTT and ADMI3 effectively reduced CYP1A1 expression induced by BaP plus 1,25(OH)₂D₃ in THP-1 cells (Fig. 4C). Thus, VDR activation is involved in effective CYP1A1 induction.

The effect of 1,25(OH)₂D₃ on CYP1A1 expression was examined in U937 cells treated with TCDD. TCDD is a high-affinity AHR ligand that is virtually not metabolized in cells (Bock and Köhle, 2006). 1,25(OH)₂D₃ increased CYP1A1 expression induced by TCDD (Fig. 4D). Next, we examined the effect of α-naphthoflavone, an AHR antagonist (Gasiewicz and Rucci, 1991), on CYP1A1 expression induced by BaP plus 1,25(OH)₂D₃ in U937 cells. The addition of α-naphthoflavone inhibited CYP1A1 induction by BaP alone and combined treatment of BaP and 1,25(OH)₂D₃ (Fig. 4E). These findings indicate that both AHR and VDR are necessary for CYP1A1 induction by BaP plus 1,25(OH)₂D₃.

VDR-RXR Binds to the CYP1A1 Promoter. VDR, pregnane X receptor, and CAR belong to the NR11 subfamily of the nuclear receptor superfamily and regulate common target genes, such as CYP3A4 (Xie et al., 2000; Thummel et al., 2001; Makishima et al., 2002). The CAR-RXR heterodimer binds to an ER8 element in the human CYP1A1 promoter (Yoshinari et al., 2010). We performed EMSAs using oligonucleotide probes containing the ER8 element and a known xenobiotoxic-responsive element (XRE) (WT in Fig. 5A). The VDR-RXR heterodimer bound to isotope-labeled XRE-ER8 (Fig. 5B). Complex formation was inhibited by addition of unlabeled XRE-ER8 (Fig. 5B). Mutation of the XRE and a proximal half-site of ER8 (MT2 in Fig. 5A) failed to exhibit competition (Fig. 5B). Addition of anti-VDR antibody induced a supershift of the VDR-RXR complex with XRE-ER8 (Fig. 5B). The results indicate that VDR-RXR binds to ER8 in the CYP1A1 promoter.

Finally, we performed ChIP assays to examine direct VDR binding to the CYP1A1 promoter in cells using anti-AHR or anti-VDR antibodies and PCR for the −379 to −693 CYP1A1 promoter region, which contains the XRE (−489 to −495) and the ER8 (−506 to −525) (Fig. 5A). Six hours after ligand addition, BaP and 1,25(OH)₂D₃ recruited AHR and VDR, respectively, to the CYP1A1 promoter in U937 cells, and combination of these compounds did not further increase the recruitment of AHR or VDR (Fig. 5C). At 24 h, VDR recruitment was slightly higher with BaP plus 1,25(OH)₂D₃ compared with treatment of 1,25(OH)₂D₃ alone (Fig. 5C). BaP and 1,25(OH)₂D₃ also induced recruitment of AHR and VDR, respectively, to the CYP1A1 promoter in THP-1 cells (Fig. 5D). Therefore, VDR regulates CYP1A1 transcription by direct binding to the promoter in monocyte/macrophage-derived cells.

Discussion

We show here that VDR activation enhanced the CYP1A1 expression and activity induced by AHR ligands in monocyte/macrophage-derived U937 cells and THP-1 cells. EMSA and ChIP assays demonstrate that VDR binds to the ER8 motif located in the proximal promoter of the human CYP1A1 gene. CAR, another NR11 subfamily nuclear receptor, also binds to the same motif and its activation induces expression of both CYP1A1 and CYP1A2 in hepatocytes (Yoshinari et al., 2010). In contrast to CYP1A1 induction, the combined treatment of BaP and 1,25(OH)₂D₃ did not induce CYP1A2 expression in U937 cells (Fig. 1). Whereas administration of BaP to mice induces Cyp1a1 mRNA levels in liver, small intestine, spleen, and bone marrow, it induces Cyp1a2 mRNA expression in liver, small intestine, and to a lesser degree in spleen, but not in bone marrow (Uno et al., 2006). Although the human CYP1A1 and CYP1A2 genes are located in a head-to-head orientation on chromosome 15 and share...
a common regulatory region (Ueda et al., 2006), transcription of these genes may be regulated by additional tissue- or cell-type-specific mechanisms. Bone marrow-derived cells may be less responsible for CYP1A2 induction. Whereas CAR activation induces expression of CYP1A1 and CYP1A2 independent of AHR (Yoshinari et al., 2010), use of an AHR antagonist, 1α-naphthoflavone, shows that the effect of 1,25(OH)2D3 on CYP1A1 induction requires AHR activation (Fig. 4). These findings indicate that VDR signaling is only effective in augmenting CYP1A1 transcription activated by AHR in monocyte/macrophage-derived cells. VDR siRNA and VDR antagonists decreased CYP1A1 expression induced by BaP plus 1,25(OH)2D3 (Fig. 4), suggesting that VDR bound to the CYP1A1 promoter is functionally active. The combination of BaP and 1,25(OH)2D3 did not further increase the recruitment of AHR or VDR to the CYP1A1 promoter (Fig. 5). Although AHR activation modifies the transcriptional activity of estrogen receptor through direct association, a direct interaction between AHR and VDR has not been detected (Ohtake et al., 2003). These findings suggest that AHR and VDR bind independently to the CYP1A1 promoter. The combination of ligands may effectively induce CYP1A1 mRNA expression through formation of a multimeric complex where AHR interacts indirectly with VDR via coregulatory proteins. Further studies are needed to elucidate the molecular mechanisms of CYP1A1-selective action of VDR on a regulatory region of the CYP1A1_CYP1A2 gene locus.

Increased expression of CYP1A1 by BaP plus 1,25(OH)2D3 resulted in enhanced BaP-DNA adduct formation (Fig. 3). BaP is first oxidized by CYP1A1 and CYP1B1 to phenols, such as 3-hydroxy-BaP and 9-hydroxy-BaP, and epoxides, such as BaP-7,8-epoxide (Shimada and Fujii-Kuriyama, 2004). We observed increased BaP hydroxylation to 3-hydroxy-BaP in cells treated with BaP and 1,25(OH)2D3 (Fig. 3), a finding consistent with increased CYP1A1 expression. BaP-7,8-epoxide is then metabolized by epoxide hydrolase to BaP-7,8-diol, which serves as a substrate for a subsequent P450-dependent oxidation, generating the toxic compound BaP-7,8-diol-9,10-epoxide (Shimada and Fujii-Kuriyama, 2004). BaP-7,8-diol is also metabolized to BaP-7,8-dione by aldoketoreductase, and BaP-1,3-, 1,6-, and 3,6-diones are thought to be formed by metabolism of BaP to phenols by P450 enzymes (Shimada, 2006). These BaP quinones are also involved in DNA adduct formation. Glutathione transferase suppresses BaP-induced DNA adduct formation by conjugation of reactive BaP metabolites (Uno and Makishima, 2009). The formation of BaP-induced DNA adducts is decreased in Hepa-1 c37 cells, a CYP1A1-deficient mutant clone of Hepa-1 cells (Maier et al., 2002). Stable transfection of a Cyp1a1 expression plasmid restores adduct formation to the level of parent Hepa-1 cells (Maier et al., 2002). Exogenous expression of the oncogenic AML1-ETO fusion protein in U937 cells up-regulates CYP1A1 expression and increases BaP-DNA adduct formation (Xu et al., 2007). These findings are similar to our result showing that CYP1A1 induction by BaP plus 1,25(OH)2D3 was
associated with BaP-DNA adduct formation (Fig. 3C). By contrast, DNA adduct formation is increased in CYP1A1-deficient mice (Uno et al., 2004), and overexpression of CYP1A1 in hepatocytes suppresses BaP-induced DNA adduct formation (Endo et al., 2008). CYP1A2 is also involved in the suppression of BaP-DNA adduct formation, but it does not cause 3-hydroxy-BaP formation (Endo et al., 2008).

CYP1A2 expression was not increased in U937 cells treated with BaP plus 1,25(OH)2D3 (Fig. 1E). Insufficient expression of CYP1A2 or other detoxifying enzyme(s) may contribute to the accumulation of BaP metabolites and to DNA adduct formation. Mono- cytes and monocyte-derived leukemia cells exhibit antimicrobial activity by producing reactive oxygen species in response to 1,25(OH)2D3 (Levy and Malech, 1991; Sly et al., 2001). BaP enhances differentiation of monocytic THP-1 cells induced by 1,25(OH)2D3 (Matsunawa et al., 2009). Increased reactive oxygen species associated with monocyte/macrophage differentiation may modify BaP metabolism and promote DNA adduct formation. In addition to monocytic differentiation, AHR activation enhances VDR-dependent expression of CYP24A1, which stimulates 1,25(OH)2D3 inactivation (Matsunawa et al., 2009). Thus, combined administration of BaP and 1,25(OH)2D3 enhances BaP-DNA adduct formation and 1,25(OH)2D3 catabolism through CYP1A1 and CYP24A1, respectively. These signaling pathways may be related to BaP-induced toxicities.

CYP1A1 is involved in the phase I metabolism of xenobiotics, such as BaP, and endogenous compounds, including estradiol and eicosanoids (Uno and Makishima, 2005; Nebert and Karp, 2008). Induced differentiation of myeloid leukemia cells by 1,25(OH)2D3 is associated with increased expression of enzymes involved in eicosanoid metabolism, such as arachidonate 5-lipoxygenase and prostaglandin-endoperoxide synthase 1 (Amano et al., 2009; Matsunawa et al., 2009). Prostaglandin production plays a role in inducing differentiation of myeloid leukemia cells (Rocca et al., 2004). CYP1A1 induced in monocyte-derived cells may be involved in monocyte/macrophage function by altering eicosanoid metabolism. Recently, AHR has been implicated in the regulation of immunity and inflammation (Kerkvliet, 2009). TCDD pretreatment enhances cholestasis-induced liver damage and proinflammatory cytokine production, phenotypes that are further exaggerated in CYP1A1/CYP1A2-null mice (Ozeki et al., 2011). The AHR-CYP1A cascade may regulate inflammatory responses in immune cells, including monocytes and macrophages. The enhancing effect of 1,25(OH)2D3 on CYP1A1 mRNA expression was also observed in breast cancer- and kidney epithelium-derived cells. Further studies are needed to elucidate the physiological role of CYP1A1 in monocytes/macrophages and other cell types.

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References