Retinoids Activate the RXR/SXR-Mediated Pathway and Induce the Endogenous CYP3A4 Activity in Huh7 Human Hepatoma Cells

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Steroid and xenobiotic receptor (SXR) or human pregnane X receptor (hPXR) dimerizes with retinoid X receptor (RXR) and regulates the transcription of genes encoding xenobiotic-metabolizing enzymes such as CYP3A4. Rifampin, the classical activator of CYP3A4, binds to SXR directly. It is unclear whether various natural and synthetic retinoids can regulate the expression of CYP3A4. To evaluate the effects of retinoids on the RXR/SXR-mediated pathway, transient transfection assays were performed on both CV-1 and human hepatoma Huh7 cells using a reporter construct containing multiple RXR/SXR consensus binding elements (an everted repeat with a 6-nucleotide spacer, ER-6). The results revealed that eight out of 13 retinoids screened significantly induced the RXR/SXR-mediated pathway in Huh7 cells. At an equal molar concentration, the acid forms (9-cis-RA, 13-cis-RA, and all-trans-RA) or aldehyde, the direct precursor of acid (9-cis-retinal and 13-cis-retinal), exhibited a greater or similar potency than rifampin. Depending on the ligands, RXR may serve as a silent or an active partner of SXR. Additionally, retinoids can increase CYP3A4 enzyme activity in Huh7 cells. To further evaluate the potential drug-drug interactions, which may be caused by retinoids, Huh7 cells were pretreated with 9-cis-RA and followed by acetaminophen. We showed that 9-cis-RA enhanced the covalent binding of N-acetyl-p-quinoneimine, a toxic intermediate of acetaminophen produced by phase I enzymes oxidation. This result suggested that drug-drug interaction might occur between 9-cis-RA and acetaminophen in human liver cells. Taken together, retinoids activate the RXR/SXR-mediated pathway and regulate the expression of CYP3A4. Thus, retinoids potentially can cause drug-drug interactions when they are administered with other CYP3A4 substrates.

Key Words: retinoids; retinoid X receptor; steroid and xenobiotic receptor; CYP3A4; liver; drug-drug interaction.

Retinoids refer to natural and synthetic derivatives of vitamin A. In humans and other omnivores, sources of vitamin A come from dietary animals and plants (Ozpolat et al., 2003). Preformed vitamin A in food, ingested as retinyl esters, and β-carotene, a major dietary carotenoid, are subsequently converted to retinol and retinal. Retinol is either esterified with long-chain fatty acids to form retinyl esters, primarily retinyl palmitate, and then mainly stored in the liver (Napoli, 1999) or converted to active metabolites, including retinol, retinoic acid (RA), and hydroxyretinotretinol (Ozpolat et al., 2003). Reversible conversion between retinol and retinal is catalyzed by alcohol dehydrogenase/reductase (Duester, 2000; Napoli, 1999) and aldo-keto reductase (Crosas et al., 2001). Cytochrome P450 (CYP) enzymes, such as CYP3A4, CYP1A1, and CYP1A2, participate in the irreversible conversion of retinal to RA (Zhang et al., 2000), including all-trans-RA, 9-cis RA, and 13-cis-RA (Mactier and Weaver, 2005; Marill et al., 2003). Retinoids are inactivated by further oxidation to form polar metabolites. CYP2C8 and CYP3A4 contribute to catabolism of RA (McSorley and Daly, 2000).

Retinoids exert multiple biological effects due to their diverse structures, chemical properties, nuclear receptor–binding affinities, molecular targets, and associated toxicity profiles (Dragnev et al., 2000). They are essential in embryogenesis, vertebrae development, vision, and homeostasis (Marill et al., 2000). Functioning as hormone-like signaling molecules, retinoids display their biological actions by binding and activating two types of nuclear receptors, retinoid X receptor (RXR) and retinoic acid receptor (RAR) (Christov et al., 2002; Collins, 2002), which are members of the nuclear receptor superfamily. RXR is activated by 9-cis-RA (Chawla et al., 2001; Mangelsdorf and Evans, 1995), whereas RAR can be activated by both all-trans-RA and 9-cis-RA (Heyman et al., 1992; Mangelsdorf et al., 1992).

RXR not only forms homodimers but also dimerizes with other nuclear receptors, such as RAR, pregnane X receptor (PXR), constitutive androstane receptor (CAR), thyroid hormone receptor, vitamin D receptor (VDR), peroxisome proliferator-activated receptor, farnesoid X receptor, and liver X receptor (Bastien and Rochette-Egly, 2004; Mangelsdorf and Evans, 1995). This may constitute the molecular basis explaining the diversity of biological actions of retinoids. RXR can serve as an active (permissive) or silent (nonpermissive) partner of other nuclear receptors (Mangelsdorf and Evans, 1995). When serving as an active partner, the heterodimer can be activated by RXR

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ligands as well as the ligand of the heterodimeric partner. Addition of both types of ligands has a synergistic effect. When RXR serves as a silent partner, RXR ligand is unnecessary and cannot activate the heterodimer. There are three RXR genes, coding for RXRa, β, and γ, all of which are able to heterodimerize with many other nuclear receptors (Mangelsdorf et al., 1992); although, there are preferences for distinct RXR subtypes to bind partner receptors in vivo (Chiba et al., 1997a,b). Among the three RXR isoforms, RXRa is dominantly expressed in the adult liver, suggesting that RXRa is the most important isoform in regulating liver function (Mangelsdorf et al., 1992).

The induction of CYP gene expression mediated by xenobiotic receptors represents an important adaptive mechanism for clearing xenobiotics (foreign compounds) from the body. One of the major xenobiotic receptors is PXR, predominantly expressed in the liver and intestine (Dragnev et al., 2000; Fuhr, 2000; Kliewer et al., 1998; Lehmann et al., 1998; Thummel and Wilkinson, 1998). In humans, PXR is also referred to as human pregnane X receptor (hPXR), or steroid and xenobiotic receptor (SXR) (Bertilsson et al., 1998; Blumberg et al., 1998). Structurally divergent xenobiotics, such as prescription drugs, herbs, pesticides, endocrine disruptors, etc., can activate PXR, leading to PXR/PXR dimerization (Blumberg et al., 1998; Jones et al., 2000; Moore et al., 2000; Willson and Kliewer, 2002; Xie et al., 2000). The RXR/PXR complex directly binds to specific DNA sequences known as xenobiotic-responsive elements, such as ER-6 (an everted repeat with a-6 nucleotide spacer) and DR-3 (a direct repeat with a-3 nucleotide spacer) localized in the regulatory regions of target genes, such as human CYP3A4 and rat Cyp3a23 (Bertilsson et al., 1998; Blumberg and Evans, 1998; Blumberg et al., 1998; Kliewer et al., 1998), and controls transcription. PXR has been identified as the master regulator of the expression of CYP3A genes, which encode the enzymes responsible for the metabolism of more than 60% of drugs used in clinics (Wang et al., 2003). The mRNA level of CYP3A is often used as an indicator of the functional status of PXR in vivo and in vitro. In addition to regulation of the CYP gene expression, activation of PXR by xenobiotics leads to trans-activation of a target gene network, which includes other phase I and phase II xenobiotic-metabolizing enzymes as well as transporters that are involved in xenobiotic detoxification pathways. PXR also plays important roles in the metabolism of endobiotics, including bile acids (Staudinger et al., 2001a,b), lipids (Bachmann et al., 2004), and bilirubin (Xie et al., 2003).

Retinoids, used as cancer therapeutic and chemopreventive agents, possess antiproliferative, differentiation-inducing, proapoptotic, and other effects that could target multiple steps in carcinogenesis (Dragnev et al., 2000). However, it is not clear whether retinoids are capable of activating the RXR/SXR-mediated pathway and then leading to potential drug-drug interactions due to CYP3A induction. Also, CYP3A participates in the catabolism of retinoids, which might be an important mechanism underlying the development of drug resistance in cancer chemotherapy using retinoids (Muidi et al., 1992). Several lines of evidence showed the correlation between levels of retinoids and CYP enzyme activity (Hauswirth, 1987; Howell et al., 1998). A recent study suggested that β-carotene and its metabolites (carotenals) may activate SXR (Ruhl et al., 2004). To evaluate the properties of different retinoids in activation of the RXR/SXR-mediated pathway, the current study is performed by measuring CYP3A4 induction at the levels of transcription and enzyme activity in a cell line–based system.

MATERIALS AND METHODS

Reagents. The resources of retinoids used in this study are listed in Table 1. These compounds are grouped into three categories: (1) carotenoids used in clinical trials or possessing antioxidant effects; (2) classical retinoids with differentiation effects or frequently used for food supplements or medication; (3) receptor-specific retinoids with anticancer effects. The action of each compound is summarized in Table 1.

Transient transfection and luciferase reporter activity assay. CV-1 and Huh7 cells were cultured in Minimum Essential Medium and Dulbecco’s Modification of Eagle’s Medium (Mediatech, VA), respectively. The media were supplemented with 10% charcoal-stripped fetal calf serum (Biomeda, CA). Cells were cultured at 37°C in 5% CO2 atmosphere with a relative humidity of 95%. Cells were plated onto 24-well plates with approximately 1 x 105 cells per well. The plated cells were cultured overnight and then transfected with different plasmids using Lipofectamine (Invitrogen, CA), according to the manufacturer’s instruction. TK(3A4)-Luc reporter construct (300 ng, provided by Drs Ronald Evans and Wen Xie), which contains three copies of the ER-6 element located in the CYP3A4 gene, was used as a reporter. Expression plasmids of RXRa (50 ng) and SXR (50 ng) were used for co-transfection when indicated. After transfection, cells were then treated with retinoids (1 x 10−5 M). Fresh medium and retinoids were provided every 24 h. A renilla luciferase expression plasmid (10 ng) was used for co-transfection as an internal control for normalization of transfection efficiency. Forty-eight hours after treatment, cells were harvested for measuring firefly and Renilla luciferase activities using the Dual Luciferase Reporter Assay System (Promega, WI).

CYP3A4 enzyme activity measurement. Huh7 cells were plated in 24-well plates (1 x 105 cells per well) and cultured overnight. The cells were then treated with retinoids (1 x 10−5 M) for 48 h. CYP3A4 enzyme activity was measured using the P450-Glo CYP3A4 enzyme activity kit (Promega, WI), according to the manufacturer’s manual.

Luminescence was measured using a single tube TD20/20 luminometer.

Northern blot analysis. Total RNA of Huh7 cells, Hep3B, and HepG2 was extracted using the guanidinium isothiocyanate method (Chomczynski and Sacchi, 1987). RNA concentration was determined spectrophotometrically. Twenty micrograms of total RNA was separated by electrophoresis in a denaturing 1.2% (wt/vol) agarose gel containing 2.2M formaldehyde. Equal loading per lane was assessed by hybridization with a β-actin cDNA probe. RNA was transferred to a nylon membrane by capillary blotting in 10× standard saline citrate and cross-linked by UV irradiation. Probe labeling and hybridization were performed as described previously (Wan et al., 2003). The differences in mRNA levels were determined by densitometry and normalized to β-actin mRNA levels.

Acetaminophen covalent binding assay. Acetaminophen (APAP) covalent binding assay was carried out according to a published method (Devalia et al., 1982). Huh7 cells cultured in 25-cm2 flasks were treated with 9-cis-RA (10μM) or solvent (DMSO) for 48 h. Cells were then treated with APAP
(10mM) mixed with 1 μCi 14C-APAP (Sigma, MO) per flask for 1 h. Cells were washed twice with ice-cold saline and lysed using M-PER reagent (M-PER mammalian protein extraction reagent, Pierce, IL). The lysate was centrifuged at 12,000 g for 10 min. The supernatants were precipitated by adding two volumes of 10% (wt/vol) trichloroacetic acid (TCA) and then centrifuged at 1000 g for 10 min. The precipitated cellular proteins were washed three times by re-suspension in 3 ml of 6.5% TCA and centrifugation at 1000 g for 3 min, followed by a final wash with 3 ml of 80% (vol/vol) methanol and centrifugation. The final pellet was suspended in 1 ml of NaOH (1 N) and heated at 70°C for 1 h. Protein concentration was determined using the Bio-Rad protein assay method (Bio-Ra, CA). The radioactivity of each sample (500 μl) was measured in Scintisafe Plus 50% (10 ml, Fisher, NJ).

**Statistical analysis.** Data are given as mean ± SD. Statistical analysis was performed using Student’s t-test or one-way ANOVA. Significance was defined by $p < 0.05$.

**RESULTS**

To determine whether retinoids are capable of activating the RXRα/SXR-mediated pathway, a panel of retinoids listed in Table 1 was screened by transient transfection assays in both CV-1 and Huh7 cells. Human RXRα and SXR expression plasmids and TK(3A4)3-Luc reporter construct were transfected into CV-1 and Huh7 cells. The transfected cells were treated with retinoids (1 × 10⁻⁵M) or rifampin (1 × 10⁻⁵M) for 48 h, followed by luciferase reporter activity assay. The results showed that eight out of 13 retinoids screened induced reporter activity in both cell lines (Fig. 1). 9-cis-RA and 9-cis-retinol were the most potent RXRα/SXR activators, showing more than 8.5-fold induction of the reporter activity in CV-1 cells. 13-cis-Retinal, 13-cis-RA, 13-cis-retinol, all-trans-RA, fenretinide, and β-carotene exerted significant inducibility, leading to 2.5- to 3.7-fold increases of luciferase activity. In Huh7 cells, 9-cis-RA, 9-cis-retinal, 13-cis-RA, and all-trans-RA exerted the most potent effects, showing greater than 4.5-fold induction of TK(3A4)3-Luc compared with the DMSO control (Fig. 1). The potency of 13-cis-retinal, 13-cis-retinol, β-carotene, and fenretinide (1.8- to 2.8-fold) was equal to or less than the equal molar of rifampin. All-trans-retinol palmitate, retinol acetate, luteine, lycopene, and TTNBP had no effects on trans-activation of the RXRα/SXR pathway in CV-1 and Huh7 cells (Fig. 1). The results indicated that retinoids can activate the RXRα/SXR-mediated pathway in a retinoid-dependent manner.

A dose-response experiment was performed to determine the potency of retinoids in activation of the RXRα/SXR-mediated pathway (Fig. 2). Eight retinoids, which had more than twofold inducibility of TK(3A4)3-Luc reporter activity in CV-1 and Huh7 cells, were selected for this experiment. Huh7 cells were used to make sure that the dose-dependent induction also takes place in liver cells. Cells were transfected with RXRα and SXR expression plasmids and TK(3A4)3-Luc reporter construct, followed by incubation with 0.1, 1, 10,

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Source</th>
<th>Features</th>
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<tr>
<td>Carotenoids</td>
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<tr>
<td>β-carotene</td>
<td>Sigma-Aldrich</td>
<td>Antioxidant. Reduction of incidence of cancer. Used in ATBC, CARET, and PHS studies (Goodman et al., 2004).</td>
</tr>
<tr>
<td>Lycopene</td>
<td>Sigma-Aldrich</td>
<td>Antioxidant. Inhibition of cell proliferation (Levy et al., 1995).</td>
</tr>
<tr>
<td>Lutein</td>
<td>US Biological</td>
<td>Reduction of the risk of colon cancer (Slattery et al., 2000).</td>
</tr>
<tr>
<td>Retinoids</td>
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</tr>
<tr>
<td>All-trans retinol palmitate</td>
<td>Sigma-Aldrich</td>
<td>Counteraction of oxidative injury (Basu and Eriksson, 2001). Used in the CARET study.</td>
</tr>
<tr>
<td>9-cis-Retinal</td>
<td>Sigma-Aldrich</td>
<td>Apoptosis in malignant cells (Fontana and Rishi, 2002). Reduction of breast cancer in premenopausal women (Torrisi et al., 2001).</td>
</tr>
<tr>
<td>13-cis-Retinal</td>
<td>Toronto Research Chem.</td>
<td></td>
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<tr>
<td>13-cis-RA</td>
<td>BIOMOL</td>
<td>RARs and RXRs specific. Reduced prostate cancer in rats (Christov et al., 2002).</td>
</tr>
<tr>
<td>Fenretinide</td>
<td>Toronto Research Chem.</td>
<td>RARs specific. Modulation of growth and differentiation. (Sporn et al., 1994).</td>
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<td>Receptor-specific retinoids</td>
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<tr>
<td>9-cis-RA</td>
<td>Sigma-Aldrich</td>
<td>RARs specific (Boehm et al., 1994).</td>
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<td>All-trans RA</td>
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<td>TTNBP</td>
<td>Sigma-Aldrich</td>
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*Note. ATBC: Alpha Tocopherol Beta Carotene Study; CARET: Carotene and Retinol Palmitate Study; PHS: Physicians Health Study; TTNBP: 4-(E-2-[5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl]-1-propenyl) benzoic acid.*
and 20µM of the selected retinoids. At the 0.1µM concentration, neither rifampin nor retinoid could trans-activate RXRα/SXR. At the 1µM concentration, only 9-cis-RA and 13-cis-RA induced the reporter activity. At the 10µM concentration, all the retinoids showed inducibility. At the 20µM concentration, all retinoids except 13-cis-retinal, 13-cis-retinol, and fenretinide demonstrated greater inducibility than equal concentration of rifampin. The results demonstrated that the activation of the RXRα/SXR-mediated pathway by retinoids is dose-dependent and some retinoids are more effective than rifampin in trans-activation of RXRα/SXR.

To dissect the role of RXRα and SXR in trans-activation of TK(3A4)3-Luc reporter, Huh7 cells were transfected with TK(3A4)3-Luc reporter construct and co-transfected with or without the expression plasmids of RXRα, SXR, or both. Transfected cells were treated with DMSO (0.1%), rifampin (1 × 10⁻⁵M), or retinoids (1 × 10⁻⁵M) for 48 h. In the absence of expression plasmids, neither rifampin nor retinoids had
an effect on trans-activation of the reporter gene, suggesting that the levels of endogenous nuclear receptors (RXRα or SXR) in Huh7 cells were too low to activate TK(3A4)-Luc (Fig. 3). RXRα alone could not mediate the trans-activation by rifampin, whereas addition of SXR or both RXRα and SXR did. This data suggested the presence of low endogenous SXR in the Huh7 cells. Addition of RXRα alone induced the trans-activation of the reporter gene when cells were treated with retinoids except 13-cis-retinol. Addition of SXR expression plasmid alone induced TK(3A4)-Luc reporter activity by all the testing retinoids again indicated the low endogenous SXR level in the Huh7 cells. When both RXRα and SXR expression plasmids were included, the induction folds were very similar to those of SXR alone (Fig. 3).

To examine the potential molecular mechanisms underlying the observed findings, we determined the relative abundance of RXRα and SXR in various hepatoma cell lines (Huh7, Hep3B, and HepG2). As shown in Figure 4, the relative levels of RXRα mRNA were higher in Huh7 and Hep3B than HepG2 cells. In contrast, SXR mRNA was not detectable in Huh7 and Hep3B cell lines, but HepG2 cells expressed high levels of SXR mRNA. These findings support the transfection data (Fig. 3) and are in agreement with the literature findings (Phillips et al., 2005).

To further investigate the interaction between retinoids and rifampin, retinoids were used in conjunction with rifampin in the transient transfection assay. Huh7 cells were transfected with TK(3A4)-Luc reporter construct, RXRα and SXR expression vectors, followed by treatments with rifampin (10 or 20μM), retinoids (10 or 20μM), and rifampin plus retinoids (10 plus 10μM). The results of luciferase reporter activity assays are illustrated in Figure 5. Six retinoids tested (9-cis-retinal, 13-cis-retinal, 13-cis-RA, 13-cis-retinol, fenretinide, and β-carotene) did not significantly further induce luciferase activity when they were added in combination with rifampin; the induction folds were similar to when retinoid was used alone (either 10 or 20μM). 9-cis-RA and all-trans-RA exerted a synergistic effect with rifampin (2.2-fold induction by rifampin, about five-, fourfold induction by 9-cis-RA and all-trans-RA, respectively, and about 12-, 9-fold induction by combination treatments, respectively). The experiments were repeated more than three times and data were reproducible. These findings suggest that RXRα may serve as an active partner of SXR when all-trans-RA and 9-cis-RA are used as ligands. It might also suggest that these two acids can activate

![FIG. 3. The role of RXRα and SXR in mediating retinoid-induced TK(3A4)-Luc activity in Huh7 cells. Huh7 cells were transiently transfected with RXRα (50 ng), SXR (50 ng), or both RXRα and SXR expression plasmids, co-transfected with TK(3A4)-Luc reporter construct (300 ng) and renilla luciferase expression vector (10 ng) as a transfection efficiency control. Rifampin (10μM) or retinoids (10μM) were incubated with transfected cells for 48 h followed by luciferase activity assays. Each value represents the mean ± SD (n = 3). *p < 0.05, compared to control (DMSO).](image1)

![FIG. 4. Basal expression levels of RXRα and SXR mRNA in three hepatoma cell lines. Total RNA was prepared from three hepatoma cell lines and the levels of RXRα and SXR mRNA were measured using northern blot as described under “Materials and Methods” section. The mRNA levels were determined by densitometry and normalized to β-actin. The relative expression levels are shown in the bar graph. Each value represents the mean ± SD (n = 3).](image2)
multiple nuclear receptor pathways to enhance the transcription of the reporter gene.

Determination of the effect of retinoids on CYP3A4 enzyme activity will provide critical information to evaluate how retinoids alter CYP3A4-mediated metabolism. To pursue this, Huh7 cells were treated with the panel of retinoids (1 × 10⁻⁵M) listed in Table 1 for 48 h, followed by CYP3A4 enzyme activity assays (Fig. 6). The results showed that six retinoids induced endogenous CYP3A4 enzyme activity by two- to sixfold in Huh7 cells. The order of inducibility is as

FIG. 5. The effect of retinoids and/or rifampin in trans-activation of TK(3A4)₃-Luc in Huh7 cells. Huh7 cells were transiently transfected with TK(3A4)₃-Luc reporter construct (300 ng), RXRα (50 ng), and SXR (50 ng) expression plasmids, and renilla luciferase expression vector (10 ng) as a transfection efficiency control. Transfected cells were treated with DMSO, rifampin (Ri, 10 and 20 μM), indicated retinoids (Re, 10 and 20 μM), and rifampin plus retinoid (10 μM for each) for 48 h. Luciferase activity assays were performed. Each value represents the mean ± SD (n = 3). *p < 0.05, compared to control (CTL).
follows: 9-cis-RA > 13-cis-RA > 9-cis-retinal > all-trans-retinoic acid > 13-cis-retinal > lycopene. Since Huh7 cells had a low basal level of SXR, rifampin was not able to induce CYP3A4 activity, which was consistent with published findings (Phillips et al., 2005) and the transfection data. Thus, the induction of CYP3A4 activity by these retinoids is likely mediated through other endogenous nuclear receptor–mediated pathway(s), such as RXRα homodimers in the Huh7 cells.

To further determine the functional consequence of retinoid-mediated CYP3A4 induction, the interaction between 9-cis-RA and APAP was monitored in Huh7 cells. Following preincubation with 9-cis-RA for 48 h, Huh7 cells were exposed to 14C-APAP (10 mM) for 1 h. Covalent binding of NAPQI, the toxic metabolite of APAP, to proteins was measured in Huh7 cells. The result indicated that 9-cis-RA significantly increased NAPQI covalent binding (Fig. 7), suggesting the possible presence of drug-drug interactions between 9-cis-RA and APAP in human liver cells.

**DISCUSSION**

The correlation between retinoids and P450 levels has been observed by several groups (Goerz et al., 1994; Howell et al., 1998; Ozpolat et al., 2003; Tsambaos et al., 1994; Westin et al., 1993). In primary culture of rat hepatocytes, retinol and RAs can induce Cyp2c7 gene expression. In addition, low abundance of Cyp2c7 was observed in vitamin A–deficient rats (Westin et al., 1993). 13-cis-RA induces hepatic microsomal P–450–dependent catalytic activities in rats (Goerz et al., 1994). Administration of a synthetic retinoid (acitretin) in rats alters the Cyp enzyme activities (Tsambaos et al., 1994). RXR-selective synthetic retinoids (LG100268 and LGD1069) increase the levels of hepatic contents of Cyp3a, 4a, and 2b1/2 when dosed to rats (Howell et al., 1998). However, the molecular mechanisms underlying these observations are elusive. The current study measured the effects of a panel of major retinoids on the RXRα/SXR-mediated pathway in CV-1 and human hepatoma cell lines. Our results demonstrated that eight out of 13 retinoids screened activated the RXRα/SXR-mediated pathway by transient transfection and the effect was either ligand- or dose-dependent. The acid forms of retinoids (9-cis-RA, 13-cis-RA, and all-trans-RA) and one of the retinaldehydes (9-cis-retinal) were among the most potent ones in activating the RXRα/SXR-mediated pathway as well as in inducing the endogenous CYP3A4 activity. 13-cis-Retinal,
13-cis-retinol, β-carotene, and fenretinide displayed similar inducibility as rifampin. Consistent with our findings, Ruhl et al. (2004) have also shown that β-carotene and its metabolites (carotenals and retinol) activate hPXR and induce the expression of hPXR target genes in human hepatoma HepG2 cells.

Since the endogenous SXR level is very low in Huh7 cells and transfection of RXXRα alone trans-activates the TK(3A4)3-Luc reporter, it is likely that other RXXR-mediated pathways can mediate the effect of retinoids in CYP3A4 induction in Hep7 cells. Xenobiotic receptors CAR and VDR cross talk with SXR. CAR and VDR also bind to the ER-6 element in the CYP3A4 promoter and regulate the gene expression (Drocourt et al., 2002; Makishima et al., 2002; Thummel et al., 2001). Whether CAR and VDR can mediate retinoid-induced CYP3A4 transcription is currently under investigation. If RXRα homodimers can induce CYP3A4 also remains to be studied. In order to elucidate the mechanism of retinoid-mediated CYP3A4 induction, a receptor-binding competition experiment needs to be done.

Based on our and others’ findings, one concern for the clinical application of retinoids is the potential drug-drug interactions due to CYP3A4 induction by retinoids. We demonstrated that 9-cis-RA significantly increased NAPQI covalent binding in Huh7 cells. This suggests that retinoids can potentially increase the susceptibility of APAP-induced hepatotoxicity in humans. In vivo studies have shown that all-trans-retinol potentiated APAP-induced hepatotoxicity in mice (Bray and Rosengren, 2001; Bray et al., 2001; Rosengren et al., 1995). However, retinol does not alter the catalytic activity and protein levels of Cyp enzymes such as Cyp1a2, Cyp2e1, and Cyp3a, involved in the metabolism of APAP in mice (Bray and Rosengren, 2001; Bray et al., 2001). Whether all the retinoids, which we have screened, can activate rodent nuclear receptors and then induce mouse Cyp enzyme activity remains to be investigated. Species-specific activation of xenobiotic receptors has been demonstrated. Typical good human SXR activators such as rifampin usually are poor activators for rodents (Barwick et al., 1996; Kocarek et al., 1995). In our study, the mechanism underlying increased APAP covalent binding by 9-cis-RA may not be relevant to the RXXRα/SXR-mediated pathway because of the low endogenous level of SXR in Huh7 cells. The bioactivation of APAP to NAPQI can be mediated by CYP3A4, CYP1A2, and CYP2E1 (Patten et al., 1993; Raucy et al., 1989). We cannot exclude the possibility that retinoids can upregulate other CYP enzymes and increase the formation of the intermediate metabolite. However, it is not clear if CYP1A2 and CYP2E1 are the nuclear receptor target genes.

In cancer therapy, resistance to therapeutic doses of retinoids rapidly occurs, which is not fully understood thus far (Marill et al., 2003). Suppression of all-trans-RA metabolism using CYP enzyme inhibitors could increase all-trans-RA levels by delaying plasma clearance in animals and humans (Marill et al., 2003). However, it is not clear whether retinoids could induce the isoforms of CYP enzymes involving retinoid metabolism in humans. CYP3A4 participates in the irreversible conversion of retinal to RA (Zhang et al., 2000) and catabolism of RA (McSorley and Daly, 2000). Our data revealed that retinoids are capable of inducing CYP3A4 expression and enzyme activity, suggesting that CYP3A4 induction by retinoids might accelerate the metabolism of retinoids themselves. This may provide a clue to explore the molecular mechanisms responsible for the development of resistance to retinoids.

In patients who receive 9-cis-RA treatment, there is a considerable interindividual variability of plasma RA concentration (Rizvi et al., 1998). Likewise, in human there is considerable interindividua difference in CYP3A4 activity (Shimada et al., 1994), suggesting the role of CYP3A4 in the metabolism of RA and in determination of retinoid treatment efficacy. The doses that we used in our experiments are clinically relevant. In cancer patients who receive all-trans-RA (88–110 mg/m2 once daily), the mean peak of all-trans-RA concentrations can reach micromolar range (Conley et al., 1997; Park et al., 2000). Following multiple dosing in healthy volunteers (Ozpolat et al., 2003), liposomal all-trans-RA displays high plasma concentrations (25.2–27.4 μM). Furthermore, the concentrations of retinol in human serum are 1.37–2.18 μM (Gueguen et al., 2002), the average concentration of liver β-carotene is 4.4 μM (Schmitz et al., 1993), and the concentration of liver retinol is 3.2 μM (Schmidt et al., 2003). Thus, it is possible that both the endogenous levels as well as the therapeutic doses of retinoids may have an impact on CYP3A4 activity, and CYP3A4 regulates retinoids metabolism, which provides a mechanism to maintain the homeostasis of retinoids in our body. The effect of retinoids on other CYP activity deserves further investigation.

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