0026-895X/06/7003-1062–1070$20.00
MOLECULAR PHARMACOLOGY
Copyright © 2006 The American Society for Pharmacology and Experimental Therapeutics
Mol Pharmacol 70:1062–1070, 2006

Vol. 70, No. 3
24125/3136677
Printed in U.S.A.

Regulation of the *CYP1A1* Gene by 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin but Not by β-Naphthoflavone or 3-Methylcholanthrene Is Altered in Hepatitis C Virus Replicon-Expressing Cells

Garret R. Anderson, Aliya Hasan, Hao Yin, Ishtiaq Qadri, and Linda C. Quattrochi

Department of Medicine (G.R.A., A.H., L.C.Q.), Department of Pediatrics (I.Q.), School of Medicine, and Department of Pharmaceutical Sciences, School of Pharmacy (H.Y.), University of Colorado at Denver and Health Sciences Center, Denver, Colorado

Received March 6, 2006; accepted June 20, 2006

ABSTRACT

Exposure to hepatitis C virus (HCV) can lead to the development of cirrhosis and hepatocellular carcinoma. To examine the effects of long-term HCV infection on hepatic cytochrome P450 1A1 (CYP1A1) expression and function, we used a human hepatoma cell line expressing the HCV subgenomic replicon (Huh.8) to evaluate *CYP1A1* induction by the aryl hydrocarbon receptor (AhR) ligand, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). In this study, we demonstrate that the induction of *CYP1A1* expression in Huh.8 cells by TCDD but not by β-naphthoflavone or 3-methylcholanthrene was significantly diminished. TCDD exposure of Huh.8 cells resulted in greater than 55% suppression of *CYP1A1* transcription compared with the parent cell line Huh7, whereas protein levels and enzyme activities were further diminished. Suppression of CYP1A1 mRNA expression in TCDD-treated Huh.8 cells was partially reversed after pretreatment with the antioxidants *N*-acetylcysteine and nordihydroguaiaretic acid, suggesting a role for oxidative stress. Induced CYP1A1 message, protein, and enzyme activity were partially restored in an Huh7 cell line expressing the HCV replicon containing a deletion in the nonstructural protein NS5A. Furthermore, adenoviral expression of NS5A in Huh7 partially suppressed TCDD-induced CYP1A1 protein and enzyme activity, implicating this protein in the mechanism of suppression. These findings demonstrate that TCDD-mediated AhR signaling is impaired in hepatocytes in which HCV is present and that NS5A alone or in the presence of other nonstructural proteins of the subgenomic replicon is in part responsible.

The cytochromes P450, a multigene family of heme-containing proteins, are responsible for the metabolism of numerous xenobiotics, including therapeutic drugs, environmental chemicals, and dietary constituents, and endogenous substrates, such as steroids and bile acids. Members of the CYP1 family include CYP1A1, -1A2, and -1B1. CYP1A1 is a highly inducible enzyme that plays a critical role in the bioactivation of certain chemicals, such as benzo[*a*]pyrene, to reactive intermediates associated with mutagenesis and carcinogenesis (Gonzalez and Gelboin, 1991). Induction is mediated by the aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor that plays a pivotal role in mediating the biological actions of a number of highly toxic chemicals, including polychlorinated-dibenzo-*p*-dioxins [of which 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD; dioxin) is a prototype ligand], polychlorinated-dibenzofurans, coplanar biphenyls, and polycyclic aromatic hydrocarbons (PAHs), such as benzo[*a*]pyrene and 3-methylcholanthrene (MC) (Burbach et al., 1992). Upon ligand binding, the cytosolic AhR migrates to the nucleus, in which it forms a dimer with Arnt (AhR nuclear translocator), which then binds to specific DNA recognition sites, referred to as Ah-response elements

This work was supported by National Institutes of Health grant GM54477.
Article, publication date, and citation information can be found at http://molpharm.aspetjournals.org.
doi:10.1124/mol.106.024125.

ABBREVIATIONS: AhR, aryl hydrocarbon receptor; Arnt, aryl hydrocarbon receptor nuclear translocator; βNF, β-naphthoflavone; HCV, hepatitis C virus; MC, 3-methylcholanthrene; NAC, *N*-acetylcysteine; NDGA, nordihydroguaiaretic acid; NS, nonstructural; PAH, polycyclic aromatic hydrocarbon; ROS, reactive oxygen species; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; UGT, UDP-glucuronosyltransferases; ER, endoplasmic reticulum; PBS, phosphate-buffered saline; bp, base pair(s); DMSO, dimethyl sulfoxide; DMEM, Dulbecco's modified Eagle's medium; NF-κB, nuclear factor κB; XRE, xenobiotic response element; RT-PCR, reverse transcription-polymerase chain reaction; EMSA, electrophoretic mobility shift assay; ANOVA, analysis of variance; DCF-DA, 2′,7′-dichlorofluorescein-diacetate; Ad-GFP, adenovirus expressing green fluorescent protein; Ad-NS5A, adenovirus expressing NS5A.

(aryl hydrocarbon response element, XRE, or dioxin response element) located in the 5′-flanking region of target genes (Denison and Whitlock, 1995). TCDD, a widespread environmental pollutant produced as the result of combustion and industrial processes, is one of the most potent agonists of the AhR and thus a powerful inducer of CYP1A1 activity.

In humans, infections from influenza, adenovirus, herpes simplex, and human immunodeficiency virus type 1 (HIV) are associated with decreases in drug biotransformation and clearance (Renton, 2004). Only a few studies have reported the effects of hepatitis infection on drug metabolism, including impairment of hepatic drug clearance in patients with HCV, as measured by metabolism of the probe drug, antipyrine (Ali et al., 1995; Jorquera et al., 2001). Studies of cytochrome P450 function have shown that CYP2A6 activity was depressed by hepatitis A (Pasanen et al., 1997) and induced by hepatitis B and C (Kirby et al., 1996), whereas Becquemont et al. (2002) found CYP3A4 and CYP2D6 activities to be significantly lower in patients with HCV than in healthy volunteers. It has been shown that changes in mRNA expression of specific P450s were linked to the progression of HCV-associated hepatocellular carcinoma (Tsunedomi et al., 2005).

HCV is a positive-stranded RNA virus that belongs to the *Flaviviridae* family. Long-term infection can lead to cirrhosis and hepatocellular carcinoma (Alter, 1995). Many factors are associated with the development of HCV-related liver damage, including exposure to such environmental agents as cigarette smoke and alcohol. However, the molecular mechanisms leading to cell injury are unclear. Long-term infection leads to cellular oxidative stress, characterized by increases in cellular levels of reactive oxygen species (ROS), suggesting that ROS may be involved in producing the damage seen in long-term HCV infection (DeMaria et al., 1996). The development of subviral systems, consisting of stable high-level expression of HCV subgenomic replicons, for the study of replication of the viral RNA in cultured cells (Lohmann et al., 1999; Blight et al., 2000) has facilitated studies on HCV replication and protein function. The subgenomic replicon is composed of six nonstructural proteins (NS) that perform various cellular functions. The NS5A protein plays a critical role in viral replication (Blight et al., 2000) and participates in numerous cellular functions, including activation of cellular transcription factors via oxidative stress (Gong et al., 2001) and activation of the endoplasmic reticulum (ER) stress pathways (Waris et al., 2002).

To our knowledge, the relationship between HCV infection and the AhR signaling pathway has not been reported. However, recent studies have investigated the role of the AhR in viral replication. Exposure of cultured cells to AhR ligands increases the replication of HIV (Yao et al., 1995; Gollapudi et al., 1996; Ohata et al., 2003) and human cytomegalovirus (Murayama T et al., 2002). The proposed mechanisms for enhanced HIV replication include TCDD-dependent generation of thiol-sensitive reactive oxygen intermediates (Yao et al., 1995), activation of NF-κB and production of tumor necrosis factor-α (Gollapudi et al., 1996), and increased gene expression through AhR binding to a putative XRE (Yao et al., 1995; Ohata et al., 2003). Adult T-cell leukemia cell lines have elevated expression of AhR and *CYP1A1*, suggesting a link between increased AhR expression and adult T-cell leukemia leukemogenesis (Hayashibara et al., 2003). Thus, the potential of infectious agents to alter AhR signal transduction pathways, including *CYP1A1* expression and function, could lead to increases in disease progression. Indeed, smoking, known to induce *CYP1A1* and hepatic *CYP1A2*, was recently shown to increase the severity of hepatic lesions in patients with long-term hepatitis C (Pessione et al., 2001; Hezode et al., 2003).

In this study, we investigated the effect of HCV on the AhR signaling pathway by examining the induction of *CYP1A1* by TCDD and other AhR ligands. We demonstrate that transcriptional activation of the human *CYP1A1* gene by TCDD, but not β-napthoflavone (βNF) or MC, is dramatically suppressed in Huh7 cells expressing the HCV subgenomic replicon. These findings will probably provide valuable insights into mechanisms of dioxin toxicity and the interactions of noninflammatory components of infectious agents on xenobiotic metabolism.

Materials and Methods

Chemicals and Reagents. Reagents were obtained as follows: TCDD was from Chemsyn Science Laboratories (Lenexa, KS). βNF, MC, *N*-acetylcysteine (NAC), and nordihydroguaiaretic acid (NDGA) were from Sigma Chemical Co. (St. Louis, MO); [α-^{32}P]dCTP, [γ-^{32}P]ATP, and poly[d(I-C)] were from GE Healthcare (Little Chalfont, Buckinghamshire, UK); and 2′,7′- dichlorofluorescein diacetate was from Alexis Biochemicals (San Diego, CA).

Cell Culture. The cell lines used in this study, Huh7, Huh.8, and Ava.1, were provided by Dr. Charles Rice (Rockefeller University, New York, NY) and are described by Blight et al. (2000). In brief, the Huh.8 cell line contains an HCV-derived expression vector stably integrated into an Huh7 background. The expression vector includes the HCV proteins NS2, NS3, NS4A, NS4B, NS5A, and NS5B linked to the antibiotic selection marker G418. The Ava.1 cell line is similar to Huh.8 but with a 47 amino acid deletion within the NS5A region, rendering this nonstructural protein nonfunctional. All cell lines were maintained as a monolayer using Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Carlsbad, CA) and heat-inactivated 10% fetal bovine serum from Hyclone (Logan, UT). G418 (Geneticin; Invitrogen) was added to a final concentration of 800 μg/ml to medium used for culturing Huh.8 and Ava.1 cell lines. For treatments, cells were exposed to TCDD, βNF, or MC at concentrations indicated in figure legends or vehicle (0.1% DMSO). NDGA was dissolved in DMSO, and NAC was dissolved in DMEM plus 25 mM HEPES and adjusted to pH 7.1 immediately before treatments.

RNA Isolation, Northern Blot Analyses, and Quantitative Real-Time RT-PCR. Total RNA was extracted from near-confluent cells using the RNeasy kit (QIAGEN, Valencia, CA). Northern blot analysis was performed by electrophoresis of total RNA (10 μg) through a 1% agarose-2.2 M formaldehyde gel followed by blotting onto a Hybond nylon membrane (GE Healthcare). CYP1A1 and β-actin cDNA probes were labeled with [α-^{32}P]dCTP using the Random Prime Kit (Invitrogen) and hybridized to the blots as described previously (Quattrochi et al., 1985). Images were quantified by phosphorimaging in the STORM 840 PhosphorImager (GE Healthcare) and using Image Quant software from GE Healthcare. For quantitative real-time RT-PCR, total RNA was treated with DNase I before analysis. Real-time RT-PCR was performed using the ABI Prism 7700 sequence detector (Applied Biosystems, Foster City, CA) with the primers shown in Table 1. Each real-time RT-PCR reaction was performed in duplicate and normalized to the ribosomal RNA levels in the same sample.

Transient Transfections and Luciferase Activity Assays. Cells were plated at a density of 125,000 cells/well in 12-well plates. Transfections were performed using Fugene 6 Transfection Reagent following manufacturer's protocol (Roche Applied Science, Indianap-

olis, IN). Five hours after transfection, DMEM containing 10% fetal bovine serum was added to each well, and cultures were incubated overnight. The culture media were removed after incubation for 24 h with the transfection reagent-DNA complexes, and the cells were then treated for 24 h with xenobiotics dissolved in DMSO. Control cells received media containing 0.1% DMSO. After treatment, cells were rinsed with phosphate-buffered saline (PBS) and luciferase assays were performed using the Dual-Luciferase Reporter Assay System (Promega, Madison, WI). Luciferase activity of cellular lysates was quantified with a Packard LumiCount luminometer. Firefly luciferase activity was determined from three independent transfections and was normalized against *Renilla reniformis* luciferase activities of the pRL-null control vector obtained from the same culture.

Western Blot Analyses. Whole-cell lysates were prepared by sonication of cell pellets in 250 mM sucrose/10 mM Tris, pH 7.5, buffer. Protein concentrations were determined using the Bradford microassay from Bio-Rad Laboratories (Hercules, CA). Western blots were prepared by electrophoresis of whole-cell lysates or nuclear extracts through 4 to 20% polyacrylamide gel electrophoresis gels and transferred to polyvinylidene difluoride membranes (MSI, Westboro, MA) overnight at 4°C. Blots were probed with antibody raised against human CYP1A1 (Santa Cruz Biotechnology, Santa Cruz, CA) or AhR and Arnt antibodies (obtained from Dr. Christopher A. Bradfield, University of Wisconsin) and subsequently with anti-actin antibody (Oncogene Research Products, San Diego, CA) to normalize the amount of protein loaded in each lane. As a positive control, each blot was run with one lane containing recombinant CYP1A1 (BD Gentest, Woburn, MA). Detection was performed using SuperSignal West Pico Chemiluminescent Substrate (Pierce Biotechnology, Rockford, IL) and visualized by UVP BioImaging Systems camera and LabWorks acquisition software (Upland, CA). Quantifications were performed using NIH Image software version 1.63 (http://rsb.info.nih.gov/nih-image/).

Isolation of Nuclear Proteins and Electrophoretic Mobility Shift Assay. Nuclear protein fractions were isolated from near-confluent cells as described by Denison et al. (1988). Cells were treated with TCDD or βNF for 24 h before extraction of nuclear proteins. Oligonucleotides were supplied by Operon Biotechnologies (Huntsville, AL). For the preparation of the CYP1A1-XRE DNA probe, the oligonucleotides 5′-CCGGCTCGCGTGAGAAGCG-3′ and 5′-CGCTTCTCACGCGAGCCGG-3′ were annealed together overnight at 37°C. The probe was labeled with ^{32}P at the 5′ ends using T4 polynucleotide kinase (Invitrogen) and [γ-^{32}P]ATP. Labeled probes were purified through TE-10 columns (Clontech, Mountain View, CA). For electrophoretic mobility shift assay (EMSA), 4 μg of nuclear extract was incubated in a DNA binding buffer containing radiolabeled probe, 10 mM Tris, pH 8.0, 75 mM NaCl, 1 mM EDTA, pH 8.0, 1 mM dithiothreitol, 2 μg of poly[d(I-C)], and 10% glycerol. Protein-DNA complexes were separated on 6% polyacrylamide gel electrophoresis 1× Tris borate-EDTA (89 mM Tris, pH 8.3, 89 mM boric acid, and 2 mM EDTA) gels. Images were quantified by phosphorimaging in the STORM 840 PhosphorImager.

P450-Glo Assay. Detection of CYP1A1 activity was performed using a P450-Glo CYP1A1 Assay Kit (Promega). The assay was performed using cultured cells according to kit specifications. Cells were plated onto 96-well plates at 30,000 cells/well. The following day, cells were exposed to TCDD for 24 h. The next day, cells were rinsed one time with PBS, medium containing a 50 μM concentration of the CYP1A1-specific substrate luciferin 6′ chloroethyl ether, was added, and cells were incubated at 37°C for 3 h. After the incubation, the reaction was terminated by adding 50 μl of luciferase detection reagent, and luciferase activity quantified with a Packard LumiCount luminometer. For each treatment group, one set of wells was assayed in the absence of substrate and values obtained subtracted from substrate wells.

Determination of ROS Production. ROS production in Huh7 and Huh.8 cells was measured spectrofluorometrically using the cell-permeable 2′,7′-dichlorofluorescein-diacetate (DCF-DA) probe. DCF-DA is converted to its fluorescent product dichlorofluorescein by ROS. Cells were plated on 96-well black plates (Nunc, Roskilde, Denmark) at 25,000 cells/well (approximately 80% confluence). The following day, media were changed to serum-free, phenol red-free DMEM, DCF-DA was added to a final concentration of 5 μM, and cells were incubated for 30 min. At the end of the incubation period, cells were rinsed one time with PBS, and media containing xenobiotics were added. Fluorescence readings were taken immediately and after various times of treatment from 15 min to 24 h using a SpectraMax Gemini EM fluorometer and SOFTmax PRO software (Molecular Devices, Sunnyvale, CA).

Recombinant Adenovirus Expression of NS5A. The construction of the recombinant adenovirus expressing NS5A is described by Qadri et al. (2004). Huh7 cells were infected with a recombinant

TABLE 1
PCR primers

Primers	Sequence	Product
		bp
CYP1A1		
Forward	5′-GAATTCAGCGTGCCACTGG-3′	69
Reverse	5′-GGCATGCTTCATGGTTAGCC-3′	
TaqMan probe	5′-CGTGAAGGTGGACATGACCCCCAT-3′	
AhR		
Forward	5′-CCTCCTTCTTGCCCTTCACC-3′	75
Reverse	5′-GGATTTGACTTGATTCCTTCAGCT-3′	
TaqMan probe	5′-CCGGTGCAGAAAACAGTAAAGCCAATCC-3′	
ARNT		
Forward	5′-TAGTGCCCTGGCTCGAAAA-3′	73
Reverse	5′-CCGCAAGGACTTCATGTGAGA-3′	
TaqMan probe	5′-CAGACAAGCTAACCATCTTACGCATGGCA-3′	

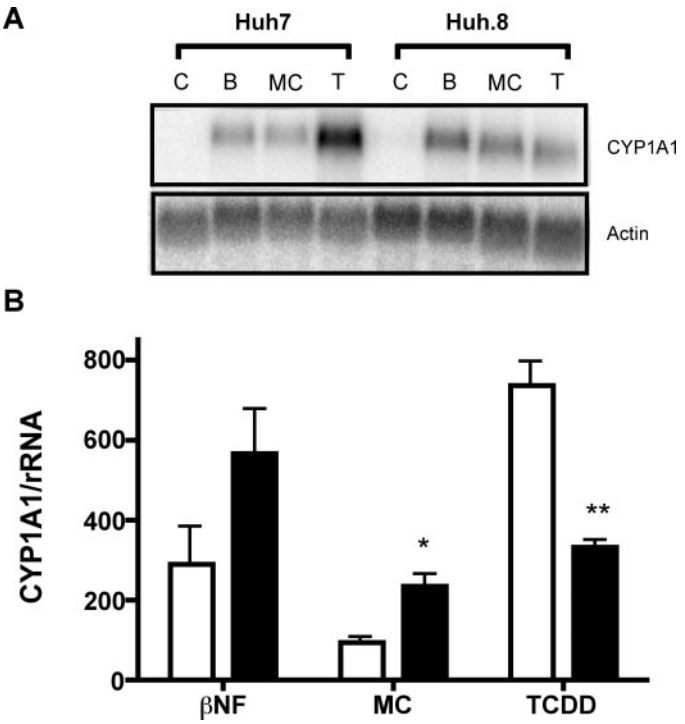

Fig. 1. Differential effects of AhR ligands on expression of CYP1A1 mRNA in the parent Huh7 cell line and the HCV replicon-expressing cell line Huh.8. Cells were treated with vehicle control (C), 10 μM βNF (B), 2 μM MC, or 10 nM TCDD (T) for 24 h. A, representative Northern blot. B, quantitative real-time RT-PCR. The histogram represents the ratio of CYP1A1 mRNA to rRNA. The values denote the mean of four independent experiments, with error bars representing S.E.M. Statistical differences between group mean values (Huh7, □, versus Huh.8, ■) were determined using the unpaired *t* test. *, $p < 0.05$; ** $p < 0.005$.

adenovirus expressing NS5A (Ad-NS5A) or expressing green fluorescent protein (Ad-GFP) as a control at a concentration of approximately 2×10^3 adenovirus particles per cell for 24 h before the addition of TCDD. The following day, media were changed, and 10 nM TCDD was added to culture plates for an additional 24 h. Cells were subsequently harvested for RNA and whole-cell protein extracts. Expression of Ad-GFP in Huh7 cells was detected using conventional fluorescence microscopy as an index of efficiency of infection.

Statistics. Statistics were performed using InStat Instant Statistics (Prism 4; GraphPad Software). Statistical differences between values were determined by a one-way ANOVA, followed by either Bonferroni or Dunnett multiple comparisons post hoc tests or Student's t test. A $p < 0.05$ is considered statistically significant.

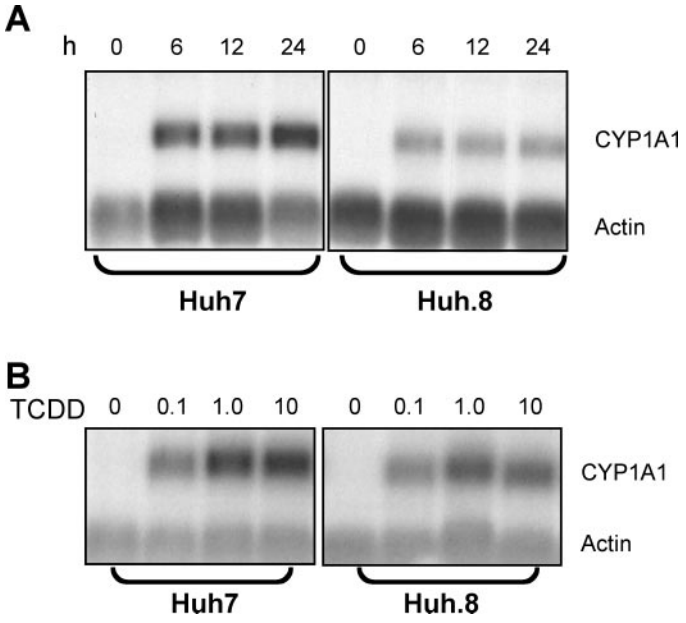

Fig. 2. Suppression of TCDD-induced CYP1A1 mRNA expression is time- and dose-dependent. A, time course: cells were treated with 10 nM TCDD for 6, 12, and 24 h. B, dose response: cells were treated with 0.1, 1, or 10 nM TCDD for 24 h. Total RNA was analyzed for CYP1A1 mRNA expression by Northern blots.

Results

Differential Effects of AhR Ligands on *CYP1A1* Gene Transcription in HCV Replicon-Expressing Cells. To examine whether the AhR pathway is affected by the presence of the HCV genomic replicon, we treated the parent cell line (Huh7) and the cells containing the HCV subgenomic replicon (Huh.8) with TCDD, βNF, or MC and assayed for the expression of CYP1A1 message. We found, as expected, that exposure of Huh7 cells to all three AhR ligands resulted in substantial induction of CYP1A1 mRNA (Fig. 1A). In contrast, exposure of Huh.8 cells to TCDD resulted in a significant reduction in CYP1A1 mRNA compared with Huh7 cells. Data from Northern blots were confirmed by quantitative real-time RT-PCR data (Fig. 1B). The expression in Huh.8 cells of TCDD-induced *CYP1A1* was diminished by 55% compared with Huh7 cells. Time-course and dose-response experiments indicated that the effects on CYP1A1 mRNA expression occur early (6 h) and at all doses tested (Fig. 2). In stark contrast, treatment of Huh.8 cells with βNF or MC resulted in an approximately 2- to 3-fold enhanced expression of CYP1A1 compared with Huh7 cells (Fig. 1).

A change in the steady-state levels of TCDD-induced CYP1A1 message suggested that the transcription of the *CYP1A1* gene was impaired in TCDD-treated Huh.8 cells. To test this, we transiently transfected a CYP1A1-luciferase plasmid (1A1Luc) containing the *CYP1A1* promoter sequences from +292 to −1612 (Postlind et al., 1993) into both cell lines and treated them with TCDD or MC. We found that luciferase activity in transfected Huh.8 cells treated with TCDD was significantly reduced compared with Huh7 cells (Fig. 3A). The decrease in reporter gene activity is consistent with the decrease in CYP1A1 mRNA levels (approximately 60%). Treatment of Huh.8 cells with MC resulted in a significant increase in luciferase activity, consistent with in-

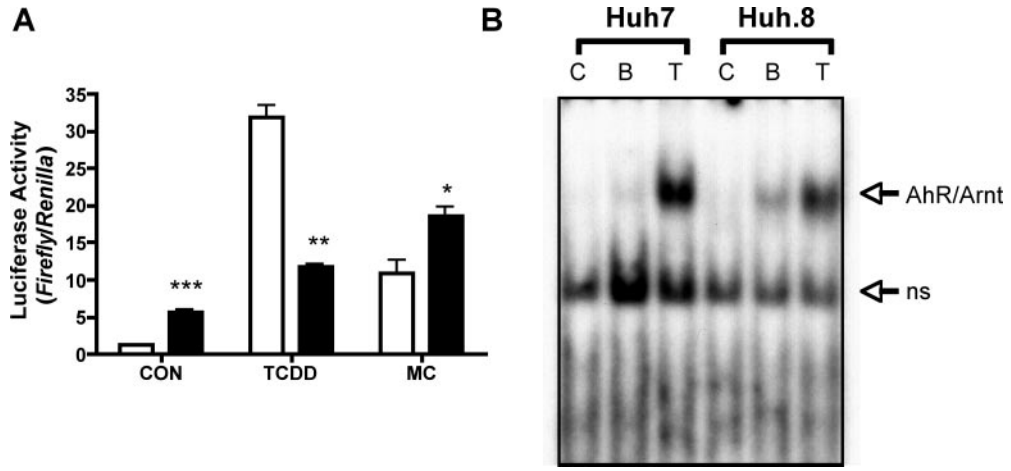

Fig. 3. Reporter gene activity and AhR binding are suppressed in TCDD-treated Huh.8 cells. A, transient transfection assays. Huh7 (□) and Huh.8 cells (■) were transiently transfected with the 1A1Luc plasmid, cells were treated with TCDD (10 nM) or MC (2 μM) for 24 h, and lysates were assayed using the Dual Luciferase Assay System as described under *Materials and Methods*. Luciferase activity is expressed as the ratio of relative light units of Firefly to *R. reniformis* activities. Values shown are for three independent transfection experiments performed in four replicates. Error bars represent S.D. Statistical differences between group mean values (Huh7 versus Huh.8) were determined using the unpaired t test. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. B, representative EMSA. Nuclear extracts from cells treated with 10 nM TCDD or 10 μM βNF for 24 h were incubated with radiolabeled human CYP1A1 XRE double-stranded oligonucleotide in a binding buffer, as described under *Materials and Methods*. Protein-DNA complexes representing the AhR/Arnt complex were quantified by phosphorimaging. Autoradiograph is representative of three nuclear preparations. ns, nonspecific complex. Free probe is not shown in this autoradiograph.

creased CYP1A1 mRNA from MC-treated cells. These findings indicate that TCDD affects AhR signaling differently than nondioxin AhR ligands in HCV replicon-expressing cells. Because βNF and MC were capable of inducing the expression of CYP1A1 mRNA, we reasoned that Huh.8 cells possessed a functional AhR. To directly test this, we performed EMSA experiments to examine AhR binding to XREs in both cell lines. We found that nuclear AhR binding was reduced in extracts from TCDD-treated Huh.8 cells compared with Huh7, but the decrease was less than the magnitude of the transcription response (approximately 30 versus 55% suppression, respectively) (Fig. 3B). We believe that these findings are not the result of unequal loading (i.e., nonspecific complex is less in Huh.8) because we obtained the same results using nuclear extracts from three different experiments. Furthermore, increased AhR binding was observed in βNF-treated Huh.8 versus Huh7 cells, consistent with mRNA data. Quantitative real-time RT-PCR of RNA from TCDD-treated cells indicated no difference in AhR mRNA expression between cell lines (Huh7, 964 ± 60 versus Huh.8, 1032 ± 347 pg/ng rRNA), whereas Arnt expression was slightly elevated in Huh.8 cells (Huh7, 735 ± 30 versus Huh.8, 1428 ± 420 pg/ng rRNA). AhR and Arnt protein levels were also similar between cell lines, as were changes in the subcellular distribution of the AhR with TCDD exposure (Fig. 4).

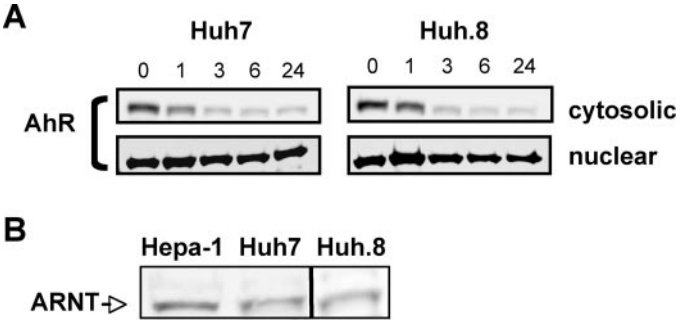

Fig. 4. AhR and Arnt protein expression is similar between Huh7 and Huh.8. A, Western blot analysis of AhR expression was performed on 30 μg of cytosolic and 15 μg of nuclear proteins prepared, as described under *Materials and Methods*, from cells exposed to TCDD (10 nM) for 1, 3, 6, and 24 h. B, analysis of Arnt expression was performed on 15 μg of nuclear protein isolated from untreated cells. Hepa-1 nuclear extract was used as a positive control.

CYP1A1 Protein Expression and Enzyme Activity Are Suppressed in Huh.8. Next, we confirmed the transcriptional data by examining the expression of CYP1A1 protein and enzyme activity in Huh7 and Huh.8 cells. Whole-cell lysates were prepared from cells exposed for 24 h to either TCDD, βNF, or MC. Increases in the level of CYP1A1 protein were observed in Huh7 cells treated with all three agents (Fig. 5A), consistent with induced message (Fig. 1). We observed a significant decrease in TCDD-induced CYP1A1 protein expression in Huh.8 cells (mean ± S.E., 13.8 ± 4.2% of Huh7) and slight increases in βNF- and MC-induced CYP1A1 protein (125.4 ± 3.1 and 211 ± 8.0%, respectively). To further characterize the regulation of the *CYP1A1* gene in Huh.8 cells, we used the P450-Glo assay to measure CYP1A1-mediated enzyme activity. CYP1A1 enzyme activity was measured directly in cultured cells after treatment with 10 nM TCDD for 24 h (Fig. 5B). The data shown in Fig. 5B revealed that CYP1A1 enzyme activity in Huh.8 cells was only 7% of Huh7 and not statistically significant from untreated cells.

Effects of ROS on TCDD-Induced CYP1A1 Expression in Huh.8 Cells. The preceding experiments demonstrated the significant effect of the HCV subgenomic replicon on AhR signaling and *CYP1A1* induction by TCDD. A potential mechanism for virus-induced down-regulation of induced *CYP1A1* expression is a change in the redox state of the cells resulting in changes in gene transcription. One explanation for our findings is that increases in cellular oxidative stress from replication of the HCV genomic replicon (Gong et al., 2001) and from TCDD-mediated production of ROS in Huh.8 cells results in a decrease in the induced transcription of the

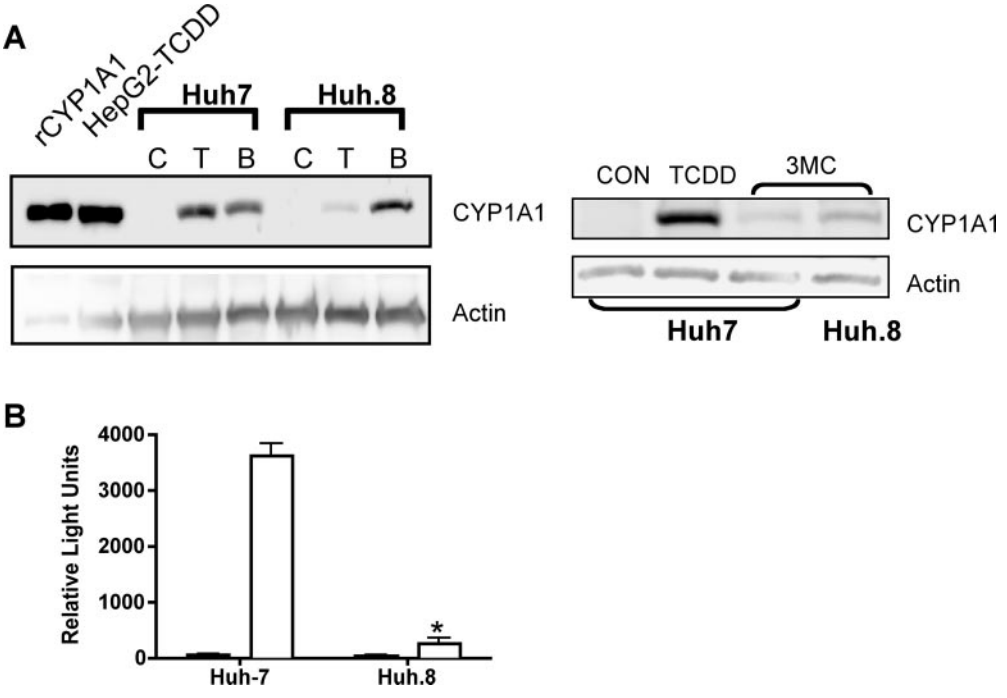

Fig. 5. CYP1A1 protein expression and enzyme activity are suppressed in the Huh.8 cells. A, representative Western blot. Cells were treated with vehicle control (C), TCDD (T) (10 nM), βNF (B) (10 μM), or MC (2 μM) for 24 h. Whole-cell lysates were prepared as described under *Materials and Methods,* and 20 μg per lane was analyzed. Blots were probed with CYP1A1 and β-actin antibodies and immune complexes were visualized using chemiluminescence. Positive controls: recombinant CYP1A1 (rCYP1A1) and lysates from TCDD-treated HepG2. Representative image shown for four to eight independent experiments. B, CYP1A1 enzyme activity was measured using the P450-Glo assay. Cells were plated in 96-well plates and treated with 10 nM TCDD for 24 h. The assay was performed using 50 μM concentration of the CYP1A1 specific substrate, and luciferase activity was measured. Data shown are the mean ± S.D. for three independent experiments performed in triplicate. Statistical differences among groups (control, ■; TCDD, □) were determined using one-way ANOVA followed by Bonferroni's test (*, $p < 0.001$).

CYP1A1 gene. To test this, we examined the role of ROS generation on *CYP1A1* transcription by preincubating Huh.8 cells with NAC, a thiol antioxidant and cysteine precursor that eliminates oxygen free radicals, and NDGA, an antioxidant and broad-spectrum inhibitor of lipoxygenase. Cells were exposed to TCDD and various concentrations of NAC or NDGA, and CYP1A1 mRNA was quantified by real-time RT-PCR (Fig. 6). The suppression of TCDD-induced CYP1A1 mRNA was partially reversed by NAC or NDGA pretreatments. CYP1A1 mRNA expression from Huh.8 cells exposed to TCDD and 20 mM NAC was 72.7 ± 9.5% of TCDD-treated Huh7 cells (Fig. 6A), whereas CYP1A1 mRNA levels from cells treated with TCDD and 15 μM NDGA was 86.6 ± 17.5% (Fig. 6B). Treatment of both cell lines with NDGA alone increased constitutive CYP1A1 message by approximately 3-fold (data not shown). Because constitutive CYP1A1 mRNA levels are elevated 10-fold in the Huh.8 cells (Fig. 9B), a further increase with NDGA treatment may have accounted for some of the changes seen with TCDD and NDGA cotreatments (Fig. 6B). Nonetheless, these results suggest a partial contribution by ROS to the suppression of TCDD-induced regulation of *CYP1A1* gene expression.

To assess the ability of TCDD itself to initiate ROS production in Huh.8 cells, we used the ROS-sensitive fluorescent probe, DCF-DA, to directly measure ROS in treated cells. Huh7 and Huh.8 cells were exposed to TCDD, MC, or βNF, and DCF fluorescence was measured between 15 min and 24 h after treatments. Increased fluorescence over control cells from inducer exposure was observed only after 6 h (data not shown). A 24-h treatment with TCDD had no effect in Huh7 cells but increased ROS production in Huh.8 by approximately 2-fold over control (Fig. 7). MC and βNF treatments increased ROS production in both cell lines (approximately 2-fold in Huh7 and 3-fold in Huh.8). ROS production from inducer-exposed cells was blocked by cotreatment with 20 mM NAC (data not shown).

The Role of NS5A on Suppression of TCDD-Induced CYP1A1 Expression in Huh.8 Cells. The HCV protein NS5A functions, alone or in the context of other HCV NS proteins, to increase ROS production through induction of oxidant stress pathways (Gong et al., 2001; Qadri et al., 2004). To examine its role in modulating induced *CYP1A1* expression, we took two approaches. First, we used a cell line, Ava.1, which expresses the HCV subgenomic replicon identical with that of Huh.8 but containing a deletion of 47 amino acids in the C terminus of NS5A, rendering this protein nonfunctional. We found treatment of Ava.1 cells with TCDD partially alleviated the down-regulation of induced CYP1A1 expression (Fig. 8A). The HCV replicon-mediated decrease in CYP1A1 mRNA levels was reversed by approximately 16% in NS5A-defective cells (left), whereas protein levels and enzyme activity were more dramatically increased, approximately 35% for protein (right) and 30% for enzyme activity (data not shown). Although TCDD-induced *CYP1A1* expression was not fully restored in the Ava.1 cell line, these findings suggested that NS5A plays a role in the suppression. To test the direct involvement of this HCV protein, Huh7 cells were infected with recombinant adenovirus expressing NS5A. We found that expression of NS5A suppressed TCDD-induced CYP1A1 protein expression by approximately 32% (Fig. 8B), which was in good agreement with results from the Ava.1 cell line.

TCDD-Induced UGT1A Expression in Huh.8 Cells. The preceding studies demonstrate an effect of HCV on the TCDD-activated AhR pathway. To determine whether this effect is specific for the *CYP1A1* gene or whether it is associated with changes in other AhR target genes, we used semiquantitative RT-PCR to analyze the expression of two UDP-glucuronosyltransferases, UGT1A1 and 1A6, known to be regulated via AhR (Auyeung et al., 2003; Sugatani et al., 2004). We found that TCDD-induced UGT1A1 mRNA is slightly suppressed, whereas 1A6 is considerably suppressed in Huh.8 cells, indicating that our observations are not specific for *CYP1A1* induction (UGT1A1, 86.7 ± 10.4%; UGT1A6, 61.0 ± 1% of Huh7) (Fig. 9). It is interesting that we found that UGT1A basal levels are substantially suppressed in Huh.8 relative to Huh7 (UGT1A1, 38.0 ± 12.2%; UGT1A6, 44.6 ± 7.2% of Huh7), results that are opposite of the constitutive expression of CYP1A1 in Huh.8 cells (Fig.

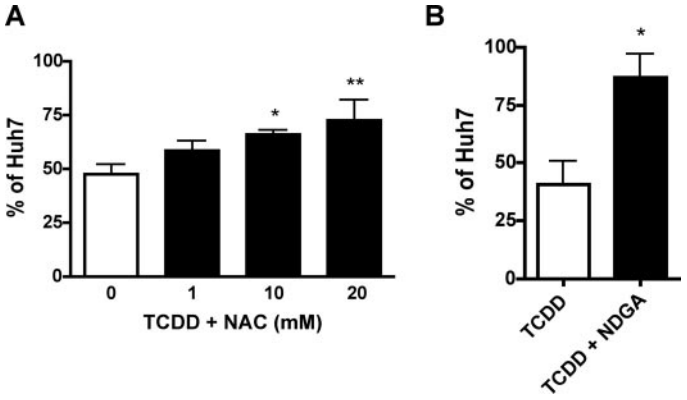

Fig. 6. The suppression of TCDD-induced CYP1A1 mRNA in Huh.8 cells is partially reversed by antioxidants. Huh.8 cells were pretreated with various concentrations of NAC (A) or 15 μM NDGA (B) for 60 min before the addition of 10 nM TCDD. After 24-h exposure to TCDD, total RNA was isolated, and CYP1A1 mRNA was assayed by quantitative real-time PCR. Shown is the mean ± S.D. of three to five experiments. For NAC treatments (■), statistical differences from TCDD control (□) were determined using one-way ANOVA followed by Dunnett's test (*, $p < 0.05$; **, $p < 0.01$). For NDGA treatment (■), statistical differences from the TCDD control (□) were determined using the unpaired t test (*, $p < 0.05$).

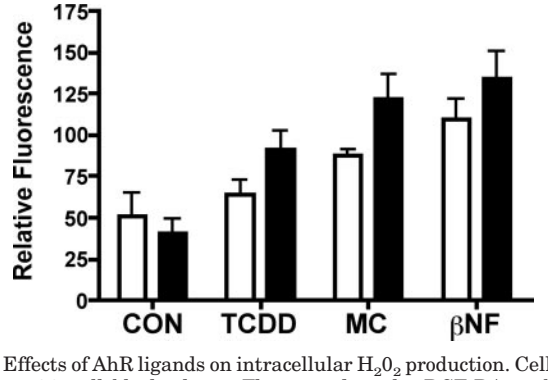

Fig. 7. Effects of AhR ligands on intracellular H_20_2 production. Cells were plated on 96-well black plates. The next day, the DCF-DA probe was added to a final concentration of 5 μM, cells were incubated for 30 min and rinsed with PBS, and media containing xenobiotics were added. Fluorescence readings were taken after various times of treatment from 15 min to 24 h. Data points shown were obtained from Huh7 (□) and Huh.8 (■) exposed to xenobiotics for 24 h. Fluorescence intensities for the DCF measurements were divided by DNA fluorescence using a DNA assay kit (Molecular Probes) to normalize cell number. The error bars represent the S.E.M. of eight replicate measurements of an individual experiment; $n = 1$.

9B). Indeed, we found constitutive CYP1A1 mRNA expression in Huh.8 cells to be increased approximately 10-fold over untreated Huh7 cells, consistent with increased reporter gene activity (Fig. 3A).

Discussion

The present study was designed to examine whether long-term hepatitis C virus infection alters the molecular mechanisms regulating induced cytochrome P450 gene expression in the absence of classic inflammatory mediators (e.g., cytokines produced by Kupffer cells). Using novel human hepatoma cell lines expressing the HCV subgenomic replicon, we found that TCDD-induced *CYP1A1* transcription and enzyme activity were dramatically suppressed. In contrast, AhR-dependent increases in *CYP1A1* expression by other AhR ligands were not suppressed but were in fact enhanced in the Huh.8 cell line that contains the HCV replicon. The AhR signaling pathway leading to induced *CYP1A1* gene expression is well described. In addition to *CYP1A1*, other AhR-responsive genes have been identified and characterized by various methods, including more recently microarray analyses (Puga et al., 2000; Fletcher et al., 2005). These studies found that in addition to numerous induced genes, dioxin

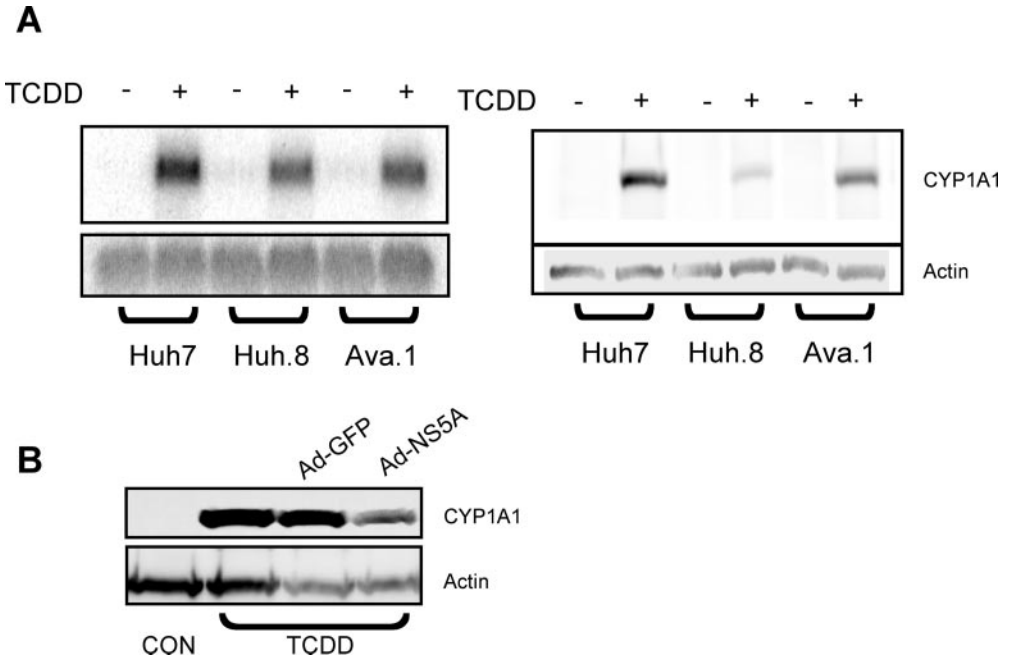

Fig. 8. Expression of the HCV protein NS5A down-regulates TCDD-induced *CYP1A1* expression in Huh7 cells. A, expression of CYP1A1 in Ava.1 cells. Cells were treated with 10 nM TCDD for 24 h, and total RNA and protein were analyzed for *CYP1A1* expression by Northern (left) and Western (right) blotting, respectively. B, adenovirus expression of NS5A. Huh7 cells were infected with 2×10^3 adenovirus particles per cell containing either Ad-NS5A or Ad-GFP as a negative control. Twenty-four hours after infection, cells were exposed to 10 nM TCDD for an additional 24 h, and 10 μg of cellular protein was analyzed for CYP1A1 expression by Western blotting.

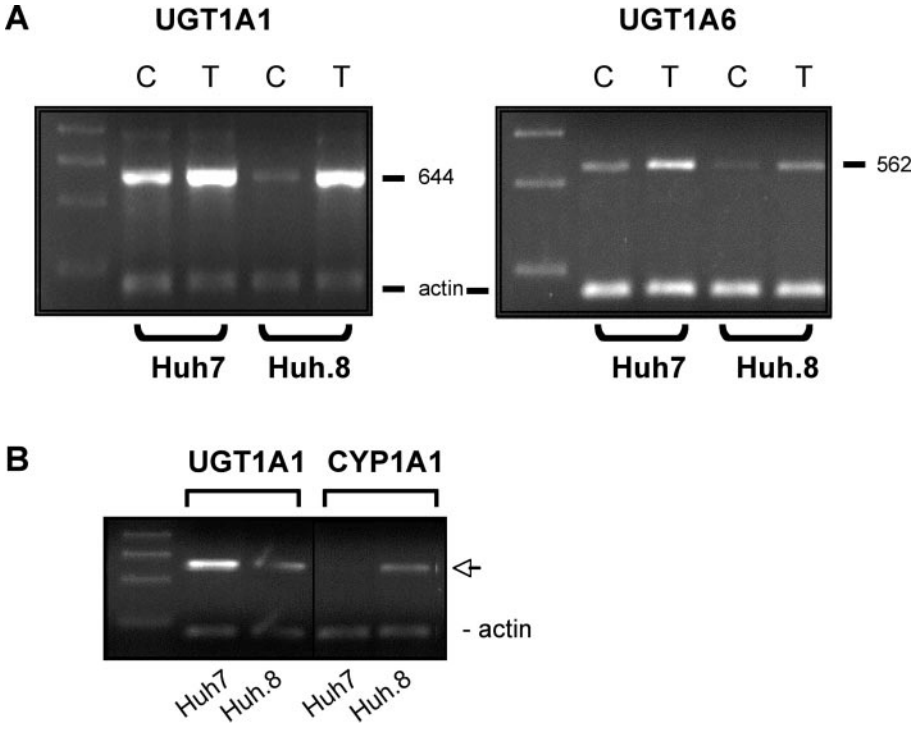

Fig. 9. A, RT-PCR products of UGT1A1 and UGT1A6 from Huh7 and Huh.8 cells. RNA was extracted from cells and used in semiquantitative RT-PCR reactions. PCR primers and conditions were exactly as described by Strassburg et al. (1997). The experiment shown is representative of three separate analyses. B, comparison of constitutive UGT1A1 and CYP1A1 mRNA expression. Arrow denotes position of amplified products. Molecular mass markers are shown in the first lane of each blot. Numbers at side of gels represent PCR product sizes.

also inhibited the expression of a number of genes. However, the molecular mechanism by which TCDD-activated AhR signal transduction leads to gene repression is still largely unknown. Most AhR agonists have been shown to function in an identical manner with respect to the activation of gene transcription; thus, our findings that βNF and MC treatment of Huh.8 cells does not suppress *CYP1A1* gene expression are unique and may provide insights into the mechanism of dioxin-induced toxicity.

The ER is the site of viral replication, and all of the HCV nonstructural proteins remain associated with this membrane. This association leads to ER stress, which involves the release of calcium from the ER, changes in the mitochondrial permeability transition pore, and increases in intracellular ROS. As a result of increases in the level of ROS, including hydrogen peroxide, superoxide radicals, and hydroxyl radicals, the transcription factors, signal transducer and activator of transcription-3 and NF-κB, are activated and migrate to the nucleus, in which they regulate target genes (Waris et al., 2002). Evidence suggests that similar increases in oxidative stress occur in Huh7 cells expressing the HCV replicon (Gong et al., 2001; Qadri et al., 2004). It has been demonstrated that increased oxidative stress down-regulates *CYP1A1* transcription (Barouki and Morel, 2001) and that CYP1A1 activity can lead to induced and sustained oxidative stress in the presence of ligands that are poorly metabolized (e.g., dioxin, polychlorinated biphenyls) (Shertzer et al., 1998; Schlezinger et al., 1999). Therefore, we reasoned that HCV replicon-induced oxidative stress, in addition to TCDD-mediated increases in ROS, could contribute to the decrease in induced *CYP1A1* expression reported here. In experiments to test this, we found that the antioxidants NAC and NDGA partially reversed the suppression (Fig. 6), indicating that suppression is mediated, in part, by an increase of ROS elicited from the HCV replicon or by a decreased capacity of the replicon-expressing cells to scavenge ROS. However, direct measurements of ROS in Huh.8 cells exposed to various AhR ligands indicated that all tested agents increase ROS production (Fig. 7). Taken together, these findings suggest that ROS may play a partial role in the down-regulation of TCDD-induced *CYP1A1* transcription in Huh.8 cells but do not explain differences in the ligand-dependent effects.

Evidence demonstrating decreased TCDD-induced *CYP1A1* transcription in Huh.8 cells suggests that the AhR signaling pathway is impaired in the presence of the HCV replicon in a ligand-dependent fashion. Additional insights into mechanisms underlying the suppression were provided by DNA binding studies. Results of EMSA (Fig. 3B) and Western blotting (Fig. 4) indicate that there is sufficient AhR present in the nucleus of TCDD-treated Huh.8 cells to drive a high level of gene transcription. These results suggest that the XRE-bound receptor may not be fully functional. Furthermore, the observation that the amount of bound AhR in TCDD nuclear extracts from Huh.8 cells is more than twice the amount from βNF extracts suggests that the decrease in AhR binding activity contributes to, but does not completely explain, the changes in *CYP1A1* transcription. This finding again emphasizes the striking differences between AhR ligands in inducing *CYP1A1* gene transcription in Huh.8 cells. The lack of complete transactivational capacity of the TCDD-activated AhR could be the result of events occurring upstream or downstream of XRE binding. HCV NS proteins are localized to the endoplasmic reticulum and therefore participate in signal transduction pathways that are initiated in the cytoplasm, including generation of ROS and activation of transcription factors (e.g., activator protein-1, NF-κB, and signal transducer and activator of transcription-3). Therefore, HCV-induced changes in the cellular redox state could alter the amount of available (i.e., functional) AhR in the cytosol. Support for this idea is provided by the recent findings that different residues in the ligand binding domain of AhR affect function of low-affinity ligands, but not TCDD (Backlund and Ingelman-Sundberg, 2004), and that phosphorylations in the 90-kDa heat shock protein modulate the formation of a functional AhR complex (Ogiso et al., 2004). These conclusions suggest the possibility that HCV-induced signaling pathways may modify residues of the cytosolic AhR, resulting in changes in ligand binding. Although we do not presently know how βNF and MC treatment of Huh.8 cells results in enhanced rather than repressed *CYP1A1* gene expression, the evidence presented here supports a ligand-dependent interaction of the AhR with the HCV replicon. Future studies of such interactions should provide important insights into AhR signaling.

In addition to changes in induced expression, we found that the presence of the HCV replicon increased the constitutive level of *CYP1A1* transcription. These findings are similar to those reported for adult T-cell leukemia, in which CYP1A1 mRNA expression was increased in the absence of exogenous ligand and was shown to be partially due to the actions of the viral transactivator protein, Tax (Hayashibara et al., 2003). The mechanism for increased CYP1A1 mRNA in Huh.8 cells may involve an endogenous AhR ligand present in HCV-replicating cells. On the other hand, other cellular factors altered by the presence of the HCV replicon could increase the rate of *CYP1A1* gene transcription. Although Western blotting was not sufficient to detect CYP1A1 protein in untreated Huh.8 cells, mRNA levels were increased at least 10-fold over untreated Huh7 cells. Constitutive expression of *CYP1A1*, not normally found in liver, may have biological implications. PAHs, known substrates for CYP1A1 metabolism, are converted to intermediate metabolites that form DNA adducts and cause toxicity. In the past, the activation of procarcinogens (e.g., PAHs) by CYP1A1 was considered a critical event leading to mutagenesis and carcinogenesis. Recent advances in genetic engineering have been able to address the in vivo significance of metabolic activation of PAHs through the use of CYP1A1-null mice (Nebert et al., 2004; Uno et al., 2004). These intriguing studies provide evidence that detoxification of procarcinogens by CYP1A1 may afford protection from toxicity depending on several factors, including target organ, route of administration, and subcellular content and localization. Thus, increased levels of constitutive CYP1A1 in HCV-infected hepatocytes might be viewed as providing a detoxification function rather than metabolic activation. On the other hand, it seems that a large portion of CYP1A1 protein may be catalytically nonfunctional in Huh.8 cells (Fig. 5B). Therefore, the possibility exists that in patients infected with HCV, cigarette smoking or exposure to other environmental agents that are substrates for CYP1A1 could be harmful, potentially leading to a more rapid progression of liver disease associated with viral infection.

Finally, TCDD suppression of gene transcription in

Huh.8 cells is not restricted to *CYP1A1* because we found similar results with UGT1A (Fig. 9). It is interesting to note that the constitutive UGT1A levels were also significantly reduced in Huh.8 cells, a finding opposite of that of *CYP1A1*. Constitutive and induced UGT1A expression are also regulated through the electrophile response element; thus, it is possible that this signal transduction pathway, which responds to oxidant stress by activating antioxidant genes, is impaired in Huh.8 cells. Indeed, inhibiting this signal transduction pathway would result in a decreased ability of the cellular defense mechanisms to detoxify ROS. Our studies demonstrate that expression and function of drug-metabolizing enzymes are substantially modified in this in vitro model of HCV. These findings may have major implications for the progression of HCV-mediated liver disease and in patient treatment, especially if CYP1A and other cytochromes P450 or phase II enzymes are similarly modified in vivo.

Acknowledgments

Quantitative RT-PCR was performed by the University of Colorado Cancer Center PCR Core Facility.

References

Ali HA, el-Yazigi A, Sieck JO, Ali MA, Raines DA, Saour J, Ernst P, Khan B, and Dossing M (1995) Antipyrine clearance and metabolite excretion in patients with chronic hepatitis C. *J Hepatol* **22:**17–21.

Alter HJ (1995) To C or not to C: There are questions. *Blood* **85:**1681–1695.

Auyeung DJ, Kessler FK, and Ritter JK (2003) Mechanism of rat UDP-glucuronosyltransferase 1A6 induction by oltipraz: evidence for a contribution of the aryl hydrocarbon receptor pathway. *Mol Pharmacol* **63:**119–127.

Backlund M and Ingelman-Sundberg M (2004) Different structural requirements of the ligand binding domain of the aryl hydrocarbon receptor for high- and low-affinity ligand binding and receptor activation. *Mol Pharmacol* **65:**416–425.

Barouki R and Morel Y (2001) Repression of cytochrome P450 1A1 gene expression by oxidative stress: mechanisms and biological implications. *Biochem Pharmacol* **61:**511–516.

Becquemont L, Chazouilleres O, Serfaty L, Poirier JM, Broly F, Jaillon P, Poupon R, and Funck-Brentano C (2002) Effect of interferon alpha-ribavirin bitherapy on cytochrome P450 1A2 and 2D6 and N-acetyltransferase-2 activities in patients with chronic active hepatitis C. *Clin Pharmacol Ther* **71:**488–495.

Blight KJ, Kolykhalov AA, and Rice CM (2000) Efficient initiation of HCV RNA replication in cell culture. *Science (Wash DC)* **290:**1972–1974.

Burbach KM, Poland A, and Bradfield CA (1992) Cloning of the AH-receptor cDNA reveals a distinctive ligand-activated transcription factor. *Proc Natl Acad Sci USA* **89:**8185–8189.

DeMaria N, Colantoni A, Fagiuoli S, Liu GJ, Rogers BK, Farinati F, Van Thiel DH, and Floyd RA (1996) Association between reactive oxygen species and disease activity in chronic hepatitis C. *Free Radic Biol Med* **21:**291–295.

Denison MS, Fisher JM, and Whitlock JP Jr (1988) Inducible, receptor-dependent protein-DNA interactions at a dioxin-responsive transcriptional enhancer. *Proc Natl Acad Sci USA* **85:**2528–2532.

Denison MS and Whitlock JP Jr (1995) Xenobiotic-inducible transcription of cytochrome P450 genes. *J Biol Chem* **270:**18175–18178.

Fletcher N, Wahlstrom D, Lundberg R, Nilsson CB, Nilsson KC, Stockling K, Hellmold H, and Hakansson H (2005) 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) alters the mRNA expression of critical genes associated with cholesterol metabolism, bile acid biosynthesis, and bile transport in rat liver: a microarray study. *Toxicol Appl Pharmacol* **207:**1–24.

Gollapudi S, Kim CH, Patel A, Sindhu R, and Gupta S (1996) Dioxin activates human immunodeficiency virus-1 expression in chronically infected promonocytic U1 cells by enhancing NF-κB Activity and production of tumor necrosis factor-α. *Biochem Biophys Res Commun* **226:**889–894.

Gong G, Waris G, Tanveer R, and Siddiqui A (2001) Human hepatitis C virus NS5A protein alters intracellular calcium levels, induces oxidative stress, and activates STAT-3 and NF-κB. *Proc Natl Acad Sci USA* **98:**9599–9604.

Gonzalez FJ and Gelboin HV (1991) Human cytochromes P450: evolution, catalytic activities and interindividual variations in expression. *Prog Clin Biol Res* **372:**11–20.

Hayashibara T, Yamada Y, Mori N, Harasawa H, Sugahara K, Miyanishi T, Kamihira S, and Tomonaga M (2003) Possible involvement of aryl hydrocarbon receptor (AhR) in adult T-cell leukemia (ATL) leukemogenesis: constitutive activation of AhR in ATL. *Biochem Biophy Res Commun* **300:**128–134.

Hezode C, Lonjon I, Roudot-Thoraval F, Mavier JP, Pawlotsky JM, Zafrani ES, and Dhumeaux D (2003) Impact of smoking on histological liver lesions in chronic hepatitis C. *Gut* **52:**126–129.

Jorquera F, Almar M, Diaz-Golpe V, Olcoz JL, Garcia-Fernandez A, and Gonzalez-Gallego J (2001) Impairment of metabolic function in chronic hepatitis C is related to factors associated with resistance to therapy. *Am J Gastroenterol* **96:**2456–2461.

Kirby GM, Batist G, Alpert L, Lamoureux E, Cameron RG, and Alaoui-Jamali MA (1996) Overexpression of cytochrome P-450 isoforms involved in aflatoxin B1 bioactivation in human liver with cirrhosis and hepatitis. *Toxicol Pathol* **24:**458–467.

Lohmann V, Korner F, Koch JO, Herian U, Theilmann L, and Bartenschlager R (1999) Replication of subgenomic hepatitis C virus RNAs in a hepatoma cell line. *Science (Wash DC)* **285:**110–113.

Murayama T, Inoue M, Nomura T, Mori S, and Eizuru Y (2002) 2,3,7,8-Tetrachlorodibenzo-p-dioxin is a possible activator of human cytomegalovirus replication in a human fibroblast cell line. *Biochem Biophy Res Commun* **296:**651–656.

Nebert DW, Dalton TP, Okey AB, and Gonzalez FJ (2004) Role of aryl hydrocarbon receptor-mediated induction of the CYP1 enzymes in environmental toxicity and cancer. *J Biol Chem* **279:**23847–23850.

Ogiso H, Kagi N, Matsumoto E, Nishimoto M, Arai R, Shirouzu M, Mimura J, Fujii-Kuriyama Y, and Yokoyama S (2004) Phosphorylation analysis of 90 kDa heat shock protein within the cytosolic arylhydrocarbon receptor complex. *Biochemistry* **43:**15510–15519.

Ohata H, Tetsuka T, Hayashi H, Onozaki K, and Okamoto T (2003) 3-Methylcholanthrene activates human immunodeficiency virus type 1 replication via aryl hydrocarbon receptor. *Microbiol Immunol* **47:**363–370.

Pasanen M, Rannala Z, Tooming A, Sotaniemi EA, Pelkonen O, and Rautio A (1997) Hepatitis A impairs the function of human hepatic CYP2A6 in vivo. *Toxicology* **123:**177–184.

Pessione F, Ramond MJ, Njapoum C, Duchatelle V, Degott C, Erlinger S, Rueff B, Valla DC, and Degos F (2001) Cigarette smoking and hepatic lesions in patients with chronic hepatitis C. *Hepatology* **34:**121–125.

Postlind H, Vu TP, Tukey RH, and Quattrochi LC (1993) Response of human CYP1-luciferase plasmids to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and polycyclic aromatic hydrocarbons. *Toxicol Appl Pharmacol* **118:**255–262.

Puga A, Maier A, and Medvedovic M (2000) The transcriptional signature of dioxin in human hepatoma HepG2 cells. *Biochem Pharmacol* **60:**1129–1142.

Qadri I, Iwahashi M, Capasso JM, Hopkin MW, Flores S, Schaack J, and Simon FR (2004) Induced oxidative stress and activated expression of manganese superoxide dismutase during hepatitis C virus replication: role of JNK, p38 MAPK and AP-1. *Biochem J* **378:**919–928.

Quattrochi LC, Okino ST, Pendurthi UR, and Tukey RH (1985) Cloning and isolation of human cytochrome P-450 cDNAs homologous to dioxin-inducible rabbit mRNAs encoding P-450 4 and P-450 6. *DNA* **4:**395–400.

Renton KW (2004) Cytochrome P450 regulation and drug biotransformation during inflammation and infection. *Curr Drug Metab* **5:**235–243.

Schlezinger JJ, White RD, and Stegeman JJ (1999) Oxidative inactivation of cytochrome P-450 1A (CYP1A) stimulated by 3,3′,4,4′-tetrachlorobiphenyl: production of reactive oxygen by vertebrate CYP1As. *Mol Pharmacol* **56:**588–597.

Shertzer HG, Nebert DW, Puga A, Ary M, Sonntag D, Dixon K, Robinson LJ, Cianciolo E, and Dalton TP (1998) Dioxin causes a sustained oxidative stress response in the mouse. *Biochem Biophys Res Commun* **253:**44–48.

Strassburg CP, Oldhafer K, Manns MP, and Tukey RH (1997) Differential expression of the UGT1A locus in human liver, biliary, and gastric tissue: identification of UGT1A7 and UGT1A10 transcripts in extrahepatic tissue. *Mol Pharmacol* **52:**212–220.

Sugatani J, Yamakawa K, Tonda E, Nishitani S, Yoshinari K, Degawa M, Abe I, Noguchi H, and Miwa M (2004) The induction of human UDP-glucuronosyltransferase 1A1 mediated through a distal enhancer module by flavonoids and xenobiotics. *Biochem Pharmacol* **67:**989–1000.

Tsunedomi R, Iizuka N, Hamamoto Y, Uchimura S, Miyamoto T, Tamesa T, Okada T, Takemoto N, Takashima M, Sakamoto K, et al. (2005) Patterns of expression of cytochrome P450 genes in progression of hepatitis C virus-associated hepatocellular carcinoma. *Int J Oncol* **27:**661–667.

Uno S, Dalton TP, Derkenne S, Curran CP, Miller ML, Shertzer HG, and Nebert DW (2004) Oral exposure to benzo[*a*]pyrene in the mouse: detoxication by inducible cytochrome P450 is more important than metabolic activation. *Mol Pharmacol* **65:**225–237.

Waris G, Tardif KD, and Siddiqui A (2002) Endoplasmic reticulum (ER) stress: hepatitis C virus induces an ER-nucleus signal transduction pathway and activates NF-kappaB and STAT-3. *Biochem Pharmacol* **64:**1425–1430.

Yao Y, Hoffer A, Chang C-Y, and Puga A (1995) Dioxin activates HIV-1 gene expression by an oxidative stress pathway requiring a functional cytochrome P450 CYP1A1 enzyme. *Environ Health Perspect* **103:**366–371.

Address correspondence to: Dr. Linda C. Quattrochi, Department of Medicine, University of Colorado at Denver and Health Sciences Center, 4200 E. 9th Avenue, Denver, CO 80262. E-mail: Linda.Quattrochi@UCHSC.edu