Induction of Mouse UDP-Glucuronosyltransferase mRNA Expression in Liver and Intestine by Activators of AhR, CAR, PXR, PPARα, and Nrf2

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Running Title: Chemical Induction of Mouse UgtS

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Non-Standard Abbreviations: AhR, aryl-hydrocarbon receptor; βNF, beta-naphthaflavone; CYP, cytochrome P450; DAS, diallyl sulfide; BHA, butylated hydroxyanisole; bDNA, branched DNA assay; CAR, constitutive androstane receptor; CLFB, clofibrate; CPFB, ciprofibrate; DEHP, diethylhexyl-phthalate; DEPC, diethyl pyrocarbamate; DEX, dexamethasone; ETHOXYQ, ethoxyquin; MEI, microsomal
enzyme inducer; Nrf2, nuclear factor erythroid 2 (NF-E2) -related factor 2; OPZ, Oltipraz; PB, phenobarbital; PCN, pregnenolone-16α-carbonitrile; PCB126, polychlorinated biphenyl 126; PPARα, peroxisome-proliferator activated receptor alpha; PXR, pregnane-X-receptor; SPR, spironolactone; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; TCPOBOP, 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene; UGT, UDP-glucuronosyltransferase.
Abstract

UGTs catalyze the addition of UDP-glucuronic acid to endo- and xenobiotics, enhancing their water solubility and elimination. Many exogenous compounds, such as microsomal enzyme inducers (MEIs), alter gene expression through xenobiotic-responsive transcription factors, namely the aryl hydrocarbon receptor (AhR), constitutive androstane receptor (CAR), pregnane X receptor (PXR), peroxisome proliferator-activated receptor alpha (PPARα), and NF-E2 related factor 2 (Nrf2). These transcription factors regulate xenobiotic-inducible expression of hepatic and intestinal biotransformation enzymes and transporters. The purpose of this study was to determine hepatic and intestinal inducibility of mouse Ugt mRNA by MEIs. Male C57BL/6 mice were treated for four consecutive days with activators of AhR (TCDD, PCB126, and β-naphthaflavone), CAR (TCPOBOP, phenobarbital, and diallyl sulfide), PXR (pregnenolone-16α-carbonitrile, spironolactone, and dexamethasone), PPARα (clofibrate, ciprofibrate, and diethylhexylphthalate), and Nrf2 (oltipraz, ethoxyquin, and butylated hydroxyanisole), respectively. Ugt1a1 mRNA expression in liver was induced by activators of all five transcription factor pathways, Ugt1a5 by Nrf2 activators, Ugt1a6 by all pathways except CAR, and Ugt1a9 by all pathways except Nrf2. Ugt2b35 mRNA in liver was induced by AhR activators and Ugt2b36 by CAR and PPARα activators. Throughout the small and large intestine, the AhR ligand TCDD increased Ugt1a6 and 1a7 mRNA. In small intestine, the PXR activator PCN increased Ugt1a1, 1a6, 1a7, 2b34, and 2b35 mRNA in the duodenum. In conclusion, chemical activation of AhR, CAR, PXR, PPARα, and Nrf2 in mouse results in induction of distinct Ugt gene sets in liver and intestine, predominantly the Ugt1a isoforms.
Introduction

Humans and other animals are constantly exposed to a variety of natural and man-made non-nutrient chemicals through the intake of air, food, drinks, drugs, and supplements. These chemicals must be eliminated from the body, as they can be toxic to cells and tissues. Therefore, detoxification mechanisms, such as biotransformation enzymes and transporters, are important for the elimination of these chemicals.

Amongst the biotransformation enzymes vital for detoxification are UDP-glucuronosyltransferases (UGTs). UGTs are a superfamily of phase-II biotransformation enzymes that catalyze the conjugation of numerous endo- and xenobiotics. Glucuronidation reactions, generally regarded as detoxification reactions, utilize the high-energy cosubstrate UDP-glucuronic acid to convert chemicals into predominantly water-soluble molecules more readily cleared by excretion through bile or urine. In humans, rats, and mice, UGTs are expressed in numerous tissues, most notably liver, small and large intestine, and kidney. Additionally, UGTs are expressed in lung, stomach, olfactory epithelium, brain, heart, and steroid-sensitive tissues such as testis, ovary, uterus, and mammary gland (King et al., 2000; Tukey and Strassburg, 2000; Shelby et al., 2003; Buckley and Klaassen, 2007). UGTs conjugate a broad range of substrates including endogenous molecules, such as bilirubin, thyroid hormones, androgens, and estrogens, as well as numerous xeno-chemicals including dietary flavinoids, phenols, non-steroidal anti-inflammatory drugs, etc (Dutton, 1980; King et al., 2000; Tukey and Strassburg, 2000).

Treatment of animals with some foreign compounds, such as microsomal enzyme inducers (MEIs), increases biotransformation enzyme activity. Molecular mechanisms
behind the induction of detoxification enzymes by MEIs have been characterized for P450s. Most notably, the orphan nuclear receptors constitutive androstane receptor (CAR) and pregnane-X-receptor (PXR) have been identified as molecular targets for xenobiotics to increase transcriptional activity of phase-I detoxification enzymes, CYP2B and CYP3A, respectively (Bertilsson et al., 1998; Honkakoski and Negishi, 1998). CAR is both constitutively active and inducible by xenobiotics, such as the anti-epileptic drug, phenobarbital, and TCPOBOP. CAR regulates gene expression through binding to the NR-1 site within the phenobarbital response element (PBRE) of the Cyp2b10 promoter (Honkakoski and Negishi, 1998; Xie et al., 2000; Yoshinari et al., 2003). PXR controls human and rodent CYP3A induction through direct ligand binding by compounds such as pregnanes, glucocorticoids, and xenobiotics (Bertilsson et al., 1998; Kliewer et al., 1998). CAR and PXR share overlapping and redundant substrate specificity and target gene induction, however, both mediate individual pathways critical for detoxification (Xie et al., 2000). Not surprisingly, human UGT1A1, the major bilirubin conjugating enzyme, is transcriptionally regulated by both PXR and CAR through DR-3 and NR-1 elements in the 5′-promoter (Sugatani et al., 2001; Xie et al., 2003). Regulation of other UGTs by CAR and PXR has been reviewed (Mackenzie et al., 2003; Zhou et al., 2005).

In addition to CAR and PXR, the nuclear receptor peroxisome proliferator activated receptor alpha (PPARα) controls transcriptional activation of UGTs. Upon ligand binding, PPARα heterodimerizes with RXR and can bind the PPAR response elements (PPREs). PPARα is responsible for the induction of CYP4A and genes involved in fatty acid β-oxidation, and is activated by fatty acids and hypolipidemic drugs, such as the fibrates (Muerhoff et al., 1992; Keller et al., 1993).
In addition to members of the nuclear receptor family, other transcription factors are involved in the transcriptional response to xenobiotic exposure, namely the aryl-hydrocarbon receptor (AhR) and NF-E2-related factor 2 (Nrf2). AhR, a helix-loop-helix protein, is liganded by polyaromatic hydrocarbons (PAHs) and dioxin, inducing expression of phase-I CYP1A enzymes as well as phase-II enzymes, glutathione-S-transferases (GSTs) and UGTs (Hankinson, 1995; Schmidt and Bradfield, 1996). Nrf2 becomes activated in response to cellular oxidative stress. Nrf2 is sequestered in the cytosol by Keap-1, where Nrf2 is targeted for ubiquitination. Upon oxidative modification of Keap-1 or phosphorylation of Nrf2, free Nrf2 (unbound to Keap-1) translocates into the nucleus, heterodimerizing with Maf proteins to induce transcription of enzymes that protect the cell from oxidative damage. Nrf2 target genes include NADPH quinone oxidoreductase (NQO1), GSTs, and UGTs (Itoh et al., 1997; Itoh et al., 2004).

To date, several studies have examined the consequences of activating the aforementioned transcription factors and their role in inducing phase-I and II biotransformation enzymes. Many studies have used human cell lines, rats, or transgenic mice to explore the mechanisms of enzyme induction. For UGT1A1, significant work has revealed numerous transcriptional activation sites in the human, and mouse, UGT1A1 promoter. Likewise, induction of UGT1A6 and 1A9 has been explored extensively in humans and rats. However, as many scientists transition to the mouse model to elicit molecular mechanisms, little data is available for the induction of mouse Ugts other than Ugt1a1. Therefore, the purpose of this study was to provide a full characterization of
mouse Ugt mRNA induction by activators of AhR, CAR, PXR, PPARα, and Nrf2 in both liver and intestine.

**Materials and Methods:**

**Chemicals:** 2,3,7,8-Tetrachloro-dibenzodioxin (TCDD) was a gift from Dr. Karl Rozman (University of Kansas Medical Center). PCB 126 was purchased from AccuStandard (New Haven, CT). Oltipraz was a gift from Dr. Steven Safe (Texas A&M University, College Station, TX). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO).

**Animals and chemical treatment:** Eight-week-old male C57BL/6 mice were purchased from Charles River Laboratories, Inc. (Wilmington, MA). Animals were housed according to the American Animal Association Laboratory Animal Care (AAALAC) guidelines. Mice were allowed food (Harlan-Teklab 8064, Madison, WI) and water ad libitum, and were acclimated to the housing facility for one week prior to treatment. Mice were treated as controls (saline, ip; corn oil, ip and oral) or an activator of AhR, CAR, PXR, PPARα, or Nrf2, as detailed in Table 1. Selection of chemical activators, and dosing regimens, were based on previous studies that examined the mRNA expression of mouse hepatic transporters and target genes of the five transcription factors examined in the current study (i.e. AhR, Cyp1a1; CAR, Cyp2b10; PXR, Cyp3a11; PPARα, Cyp4a14; and Nrf2, Nqo1) (Cheng *et al*., 2005). Doses were selected based on the ability to effectively activate each of the five transcription factors and induce target gene mRNA expression in mouse liver. Mice were dosed once daily for 4 consecutive days in a volume of 5 mL/kg and tissues were harvested on day 5. Liver,
duodenum, jejunum, ileum, and colon were removed and immediately frozen in liquid nitrogen and stored at –80°C. Intestine was rinsed with saline prior to freezing.

**Total RNA Isolation:** Total RNA was extracted from each tissue using RNA-Bee Reagent (Tel-Test Inc., Friendswood, TX) according to the manufacturer’s protocol. RNA was quantified by UV spectrophotometry at 260/280nm and diluted to 1μg/μl in diethyl pyrocarbamate (DEPC)-treated water. RNA samples were analyzed by formaldehyde-agarose gel electrophoresis, and integrity was confirmed by visualization of 18S and 28S rRNA bands.

**Branched DNA Signal Amplification (bDNA) Assay:** Individual mouse Ugt mRNA transcripts were detected using the QuantiGene® bDNA signal amplification assay (Panomics, Fremont, CA). Probe sets for mouse Ugt transcripts were previously described (Chen et al., 2003; Buckley and Klaassen, 2007). Probes for Cyp1a1, Cyp2b10, Cyp3a11, Cyp4a14, and Nqo1 were also previously described (Cheng et al., 2005). The bDNA assay was performed as described and validated previously (Hartley and Klaassen, 2000). Briefly, capture extender, label extender and blocker probes were combined and diluted into lysis buffer. Total RNA (1 μg/μl; 10 μl) was added to each well of 96-well plates containing 50 μl of capture hybridization buffer and 50 μl of diluted probe set, and allowed to hybridize at 53°C overnight. Plates were cooled to 46°C and rinsed twice with wash buffer. Amplifier reagent (100 μl), diluted 1:1000 in amplifier/label probe buffer, was added to each well and incubated at 46°C for 1 hr. Plates were rinsed again with wash buffer and label reagent (100 μl), diluted 1:1000 in amplifier/label probe buffer, and incubated at 46°C for 1 hr. Plates were rinsed in wash buffer, and substrate reagent (100 μl) was added to each well. Alkaline phosphatase
luminescence was activated by the addition of dioxetane substrate reagent. Plates were incubated for 1 hr at 37°C, and luminescence was quantified with a Quantiplex 320 bDNA Luminometer (Panomics, Inc., Fremont, CA). Analysis of luminescence from the 96-well plates was performed by Quantiplex Data Management Software version 5.02 (Panomics, Inc.). Luminescence for each well is reported as Relative Light Units (RLU) per 10 μg total RNA.

**Statistical Analysis:** Data are presented as a mean with bars representing standard error of the mean (S.E.M.). Statistical differences between control and treatment groups were determined by a one-way analysis of variance (ANOVA) followed by Duncan’s multiple range post-hoc test. Statistical differences between a control group and a single treatment group were determined by a two-tailed Student’s t-Test. Asterisks represent statistical differences (p ≤ 0.05) in mRNA levels between control and treatment groups.

**Results**

**Activation of Transcription Factor Pathways.** To determine whether treatments (Table 1) sufficiently and effectively activated each specific transcription factor pathway, induction of mRNA expression target genes for each pathway was determined by bDNA in liver (Figure 1). Cyp1a1 mRNA, a marker of AhR activation, was induced in liver by TCDD, PCB 126, and β-NF. The CAR target gene, Cyp2b10, was increased by TCPOBOP, DAS, and PB. PXR target gene, Cyp3a11, mRNA was induced by PCN, SPR, and DEX. All PPARα activators, CLFB, CPFB, and DEHP,
increased expression of Cyp4a14, and all Nrf2 activators OPZ, ETHOXYQ, and BHA increased Nqo1 mRNA expression.

**Ugt1a mRNA Induction in Mouse Liver.** Figure 2 illustrates the effects of all 15 treatments on the Ugt1a genes that are basally expressed in liver of C57BL/6 mice, in particular Ugt1a1, 1a5, 1a6, and 1a9 (Buckley and Klaassen, 2007). In liver, each of the aforementioned genes were induced by at least one class of transcriptional activator. Ugt1a1 mRNA expression was increased by at least 2 of 3 activators of each transcriptional pathway. AhR ligands TCDD, PCB126, and β-NF increased Ugt1a1 expression 47 to 81% as did CAR activators TCPOBOP and PB, both by 68%. Ugt1a1 mRNA expression was increased by PXR activators PCN and SPR, 47 and 86%, respectively, but was decreased by DEX to 47% of control. PPARα activators CPFB and DEHP produced the most marked increase in Ugt1a1 expression, 90 and 108%, respectively. All three Nrf2 activators, OPZ, ETHOXYQ, and BHA, increased Ugt1a1 expression in liver 40, 66, and 78%, respectively.

Ugt1a5 mRNA expression was increased by two Nrf2 activators, OPZ and ETHOXYQ, 137 and 172%, respectively. Ugt1a5 mRNA was increased by β-NF and SPR (Figure 2).

Ugt1a6 mRNA was increased by at least 2 of 3 activators of AhR, PXR, PPARα, and Nrf2 (Figure 2). AhR ligands TCDD, PCB126, and β-NF resulted in the most profound increases in Ugt1a6 mRNA expression of 197, 133, and 89%, respectively. Ugt1a6 was modestly increased by one CAR activator, DAS, in liver by 71%. PXR activators PCN and SPR (54 and 64%) increased Ugt1a6 mRNA as well as did PPARα.
activators CLFB, CPFB, and DEHP (112, 74, and 95%), and Nrf2 activators, OPZ and BHA (67 and 116%).

Ugt1a9 mRNA was induced by at least 2 of 3 activators of AhR, CAR, PXR, and PPARα (Figure 2). AhR ligands again produced the most marked increases of 504, 313, and 265% for TCDD, PCB126, and β-NF, respectively. Ugt1a9 mRNA expression was also increased by CAR activators TCPOBOP and DAS (86 and 96%), PXR activators PCN, SPR, and DEX (94, 138, and 283%), as well as PPARα activators CLFB and CPFB (148 and 142%).

Ugt1a2 1a7, and 1a10 are extrahepatic mouse Ugt isoforms expressed at very low levels in untreated liver. The effects of the 15 chemicals on mRNA levels of these Ugts are illustrated in Figure 3. None of the extrahepatic genes exhibited overall induction by any class of compound, defined as induction by at least 2 of 3 activators of a specific transcriptional pathway. However, Ugt1a2 mRNA expression in liver was increased by treatment with SPR.

**Ugt2b mRNA Induction in Mouse Liver.** The effects of the chemical activators of transcription factors on mouse Ugt2b mRNA expression are shown in Figure 4. Ugt2b1 mRNA expression was not induced by any treatment; however, Ugt2b1 was decreased by two Nrf2 activtors, OPZ and BHA, as well as the AhR ligand TCDD. Ugt2b35/37/38 was not increased or decreased by any chemical treatment. Ugt2b34 mRNA was increased by two compounds, TCPOBOP and PCN, by 106 and 65%, respectively. Ugt2b35 mRNA was increased by 2 of 3 activators of AhR, namely TCDD and PCB126, by 71 and 99%, respectively. DAS and PCN also increased Ugt2b35 mRNA expression in liver. Lastly, Ugt2b36 mRNA was induced by all three activators
of CAR and PPARα, ranging from 55-73%. Additionally, Ugt2b36 mRNA expression was increased by SPR in liver.

**Ugt2a3 and Ugt3a1/2 mRNA Induction in Mouse Liver.** Ugt2a3 and Ugt3a1/2 are the two non-Ugt1a or -2b Ugt genes expressed in mouse liver. Mouse hepatic Ugt2a3 was decreased by two activators of both AhR and CAR (Figure 5). TCDD and β-NF as well as TCPOBOP and DAS decreased Ugt2a3 mRNA 35-40% in liver. Ugt3a1/2 mRNA was decreased about 45% by two CAR activators, TCPOBOP and DAS. Neither Ugt2a3 nor 3a1/2 was increased by any of the 15 treatments.

**Induction of Ugt mRNA in Mouse Intestine.** Intestines were collected from male mice treated with the prototypical activator of each transcription pathway, TCDD, TCPOBOP, PCN, CLFB, and BHA. Small intestine was divided into thirds and classified as duodenum, jejunum, and ileum (proximal to distal). Colon was also collected. Ugt mRNA expression was quantified in all four portions of intestine from mice treated with each of the five aforementioned inducers and controls (Figure 6). Intestinal RNA samples within a single treatment group (n=5 mice/group) were pooled into a single sample before performing the bDNA assay on the six Ugt genes previously detected in mouse intestine, namely Ugt1a1, 1a6, 1a7, 2a3, 2b34, and 2b35 (Buckley and Klaassen, 2007). From the results illustrated in Figure 5, two major trends were observed: 1) TCDD increased Ugt1a6 and 1a7 in duodenum, jejunum, ileum, and colon, and 2) PCN increased all six Ugt genes examined in duodenum. For these groups, the bDNA assay was repeated using individual rather than pooled samples (Figure 7).

Ugt1a6 mRNA expression in the duodenum, jejunum, ileum, and colon was increased by TCDD, showing 106, 213, 329, and 67% increases, respectively (Figure
7A). Ugt1a7 mRNA expression was increased by TCDD in intestine as well, showing 132, 131, 240, and 70% increases, from duodenum to colon (Figure 7B). PCN increased mRNA expression of Ugt1a1, 1a6, 1a7, 2b34, and 2b35 in duodenum (Figure 7C); however, PCN induced increases of the aforementioned transcripts in duodenum were modest, ranging from 50 to 130%.

**Discussion**

UGTs, as well as other phase-II biotransformation reactions, such as glutathione conjugation and sulfation, are essential for defense from exposure to xenobiotics as well as endogenous waste products and signaling molecules. Conjugation and detoxification, or signal termination in the case of steroids, is critical to prevent cellular damage from potentially toxic chemicals. Under normal physiological conditions, cells express a homeostatic or basal level of detoxification enzymes required for defense against endogenous byproducts and oxidative stress formed during normal cellular metabolism. However, exposure to xenobiotics requires cells to enhance their defense, thus resulting in an increased expression and functional utilization of detoxification enzymes. Xenochemicals and some endogenous molecules induce enzyme expression by acting as ligands or activators of cellular pathways leading to increased gene transcription, most notably through nuclear receptor mechanisms. In this study, the effects of prototypical activators of xenobiotic-inducible transcriptional pathways on mouse Ugt gene expression were determined. Activators of AhR, CAR, PXR, PPARα, and Nrf2 were used to determine which transcriptional pathways can induce individual Ugt isoforms upon xenobiotic exposure.
The most common transcription factors associated with xenobiotic metabolism are PXR and CAR, whose activation by foreign and endogenous chemicals results in the subsequent increase of mRNA expression of drug-metabolizing enzymes and transporters. PXR activators induced mRNA expression of hepatic Ugt1a1, 1a6, and 1a9 in mice. These results are not surprising. Our laboratory previously reported that Ugt1a1 and 1a9 mRNA induction by PCN is PXR-dependent using PXR-null mice (Chen et al., 2003). Ugt1a6 was not significantly induced in the aforementioned study, but differences in dose and route (food vs. ip injection) may explain the differential induction in Ugt1a6 in liver. Mice overexpressing human PXR exhibited significant induction of Ugt1a1 and 1a6 mRNA and protein, likewise, Ugt1a1 mRNA is increased in humanized PXR mice treated with rifampin (Sonoda et al., 2003; Xie et al., 2003). In rats, UGT1A1 mRNA is also increased by PXR ligands (Shelby and Klaassen, 2006). Additionally, human UGT1A1 is induced by rifampin in HepG2 cells overexpressing PXR, and confirmed by EMSA and site-directed mutagenesis that PXR/RXR binds to a DR-3 in the hUGT1A1 promoter (Xie et al., 2003). Additionally, human UGT1A3, 1A4, 1A6, and 1A9 are induced by activation of PXR (Zhou et al., 2005).

In the current study, PCN induced several mouse Ugt genes in duodenum. PCN induces glucuronidation activity in rat intestine (Goon and Klaassen, 1992). More recently, humanized UGT1A transgenic mice revealed that PCN induces UGT1A1 and 1A4 in small intestine at the mRNA and protein level, resulting in increased glucuronidation activity after PCN treatment (Chen et al., 2005). In the present study, mouse Ugt1a1, 1a6, 1a7, 2b34, and 2b35 mRNA were increased by PCN in the
duodenum. The same trend was not observed in more distal portions, specifically the jejunum, ileum and colon.

A previous report suggests DEX induction of human UGT1A1 in HepG2 cells occurs through mechanisms involving CAR, PXR, and GR in the promoter region, and further suggests a role for the transcriptional co-activator GRIP-1 in mediating the response (Sugatani et al., 2005). In the present study, mouse Ugt1a1 mRNA was decreased by DEX treatment. More studies should be performed to determine whether Ugt1a1 mRNA repression by DEX in mice is species, strain, or dose-dependent.

Xenobiotics can also activate CAR, enhancing transcription of CYP2B and UGTs. CAR mediates PB induction of human UGT1A1 in HepG2 cells by binding to a NR1 site of the PBREM located within the UGT1A1 promoter region (Sugatani et al., 2001). In rat liver, CAR activators increase mRNA expression of UGT1A1, 1A5, 1A6, 2B1, and 2B12 (Shelby and Klaassen, 2006). In mice over-expressing CAR, Ugt1a1 is up-regulated (Saini et al., 2005). Additionally, TCPOBOP induction of mouse Ugt1a1 in vivo is ablated in CAR-null mice (Huang et al., 2003). In this study, activators of CAR increased mRNA expression of not only Ugt1a1, but Ugt1a9 and 2b36 as well. Induction of bilirubin-conjugating Ugt1a1 in liver supports increased bilirubin clearance in CAR-activated mice (Huang et al., 2003). Interestingly, the mouse Ugt2b1 was not increased by CAR activators, contrary to its rat ortholog, UGT2B1 (Shelby and Klaassen, 2006). This observation suggests differential induction between species, resulting from perhaps differences within proximal promoter elements. In contrast to CAR activators increasing expression of a number of Ugts, the activators decreased the mRNA expression of
Ugt2a3 and 3a1/2. The importance of the repression of these two enzymes remains a mystery due to the lack of information regarding substrate specificity of Ugt2a3 and 3a1/2.

Activation of AhR by PAHs and TCDD and the subsequent induction of UGT1A enzymes in liver and intestine of humans, rats, and mice is well documented. Up-regulation of human UGT1A1 in liver by TCDD and β-NF occurs via activation of AhR and subsequent binding to the XRE in the UGT1A1 promoter region (Yueh et al., 2003). In Caco-2 cells, TCDD induces human UGT1A6 and 1A9 (Munzel et al., 1999). Humanized UGT1A mice revealed hepatic induction of UGT1A1, 1A3, 1A4, 1A6, and 1A9 by TCDD, as well as UGT1A3 and 1A10 in small intestine, and UGT1A5 and 1A7 in large intestine (Chen et al., 2005). In rats, TCDD and 3-MC induce UGT1A1 and 1A6 through a XRE, whereas the mechanism of TCDD induction of UGT1A7 has not been characterized (Munzel et al., 1994; Emi et al., 1995; Mackenzie et al., 2003). Rat UGT1A3 mRNA is increased by PCB126 and β-NF (Shelby and Klaassen, 2006). In mice, Ugt1a6 mRNA induction by TCDD in WT mice is abolished in AhR-null mice (Nishimura et al., 2005).

The present study further illustrates the induction of Ugt1a family members by AhR ligands in mice. AhR ligands increase mRNA expression of Ugt1a1, 1a6, 1a9, and 2b35 in mouse liver. Similar to induction of human UGT1A6 and rat UGT1A6 and 1A7 by AhR ligands, TCDD induced mouse Ugt1a6 and 1a7 in the small intestine (duodenum, jejunum, and ileum) and colon. Interestingly, AhR ligands decreased Ugt2a3 in mouse liver, suggesting both positive and negative regulation by AhR in liver, likely due to differences in proximal promoters.
PPARα activation by the hypolipidemic fibrate drugs alters lipid metabolism via fatty acid β-oxidation. In addition, PPARα activation by fibrates induces expression of the bile acid-conjugating UGT, UGT2B4, in human hepatocytes (Barbier et al., 2003b). Analysis of Ugt2b mRNA and protein induction by fibrates in PPARα-null mice indicates that PPARα regulates inducible and basal gene expression (Barbier et al., 2003a). In the present study, Ugt2b36 was the only Ugt2b family member induced by PPARα ligands in mouse liver. Previously, mouse hepatic Ugt1a9 was shown to be inducible by both PPARα and PPARγ ligands, which is ablated in PPARα-null mice (Barbier et al., 2003c). In the present study, Ugt1a9 mRNA was induced by PPARα ligands. In addition to Ugt1a9 and 2b36, PPARα activators increased mRNA expression of Ugt1a1 and 1a6 in mouse liver. Barbier et al. (2003b) suggested that glucuronidation of catecholestrogens, fibrates, and anti-diabetic drugs may be a response to regulate tissue concentrations of such drugs and thereby altering or terminating their hormonal signals.

Nrf2 activation is a product of the cellular response to oxidative or electrophilic stress, inducing some phase-I and -II detoxification enzymes, including UGTs. Nrf2 activators increased mRNA expression of Ugt1a1, 1a5, and 1a6 in mouse liver. In contrast, Nrf2 activators repressed expression of Ugt2b1 in liver. Previous studies show that in rats UGT1A6 mRNA and glucuronidation of 1-naphthol (a UGT1A6 substrate) is increased by BHA, ETHOXYQ, and OPZ (Goon and Klaassen, 1992; Buetler et al., 1995; Auyeung et al., 2003; Shelby and Klaassen, 2006). Additionally, in Nrf2-null mice, basal glucuronidation activity and Ugt1a6 mRNA is reduced as well as Ugt1a6 induction by OPZ is ablated (Enomoto et al., 2001; Ramos-Gomez et al., 2001). In mouse small intestine, sulforaphane increases Ugt1a6 in a Nrf2-dependent manner.
(Thimmulappa et al., 2002). In the present study, the Nrf2 activator BHA did not increase any Ugt mRNA expression in small or large intestine. Dose and route of exposure of the Nrf2 activators BHA and sulforaphane may explain the observed differences.

There are seven mouse Ugt1a isoforms, four of which are abundantly expressed in untreated mouse liver, notably Ugt1a1, 1a5, 1a6, and 1a9 (Buckley and Klaassen, 2007). The present study indicates that activation of the five transcriptional pathways only affects the four aforementioned Ugt1a genes. Ugt1a2, 1a7, and 1a10 were not induced by any class of inducer; only one compound, SPR, increased mRNA expression of these genes. However, in rats, UGT1A3 and 1A5 mRNA are expressed at low levels in livers of control animals, but are readily induced by activators of AhR, CAR, PPARα, and Nrf2 (Shelby and Klaassen, 2006).

In conclusion, activation of AhR, CAR, PXR, PPARα, or Nrf2 results in an increase of three or more mouse Ugt genes in liver. Transcriptional activation of Ugt1a1, 1a6, 1a9, and 2b36 is increased by two or more of the five xenobiotic-inducible transcriptional pathways. AhR ligands produced the highest amplitude of gene induction in liver, with Ugt1a6 and 1a9 being induced 3- to 5-fold higher than controls. In duodenum, PCN, a prototypical rodent PXR inducer, increased expression of five Ugt genes, however, that effect was not observed in more distal regions of intestine (jejunum, ileum, and colon). TCDD, an AhR ligand, increased expression of Ugt1a6 and 1a7 in all portions of the small intestine (duodenum, jejunum, and ileum) and colon. Taken together, these data reveal that activation of a single transcriptional pathway leads to induction of numerous target genes within the mouse Ugt family. These data provide a
significant background for understanding the targets of AhR, CAR, PXR, PPARα, and Nrf2 in liver and intestine. Drugs or xenobiotics found to activate one of these transcription factors will influence Ugt gene expression, and most likely glucuronidation of specific substrates, whether endogenous or exogenous. Likewise, drugs conjugated by an inducible Ugt isoform may encounter altered metabolism and disposition.
References


Footnotes:


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Figure 1. Messenger RNA expression of Cyp1a1, Cyp2b10, Cyp3a11, Cyp4a14, and Nqo1 by the bDNA assay in male mouse livers treated with activators of AhR, CAR, PXR, PPARα, and Nrf2, respectively (n = 5/treatment). Data are expressed as fold induction compared with vehicle controls. Values are expressed as mean ± S.E.M.. Asterisks (*) indicate statistically significant differences between control and treatment groups (p ≤ 0.05).

Figure 2. Messenger RNA expression of Ugt1a1, 1a5, 1a6, and 1a9 in livers of male mice treated with three activators of AhR, CAR, PXR, PPARα, and Nrf2 (n = 5/treatment) by the bDNA assay. Data are expressed as percent control. Values are expressed as mean ± S.E.M.. Asterisks (*) indicate statistically significant differences between control and treatment groups (p ≤ 0.05).

Figure 3. Messenger RNA expression of Ugt1a2, 1a7, and 1a10 in livers of male mice treated with three activators of AhR, CAR, PXR, PPARα, and Nrf2 (n = 5/gender) by the bDNA assay. Data are expressed as percent control. Values are expressed as mean ± S.E.M.. Asterisks (*) indicate statistically significant differences between control and treatment groups (p ≤ 0.05).

Figure 4. Messenger RNA expression of Ugt2b1, 2b5/37/38, 2b34, 2b35 and 2b36 in livers of male mice treated with three activators of AhR, CAR, PXR, PPARα, and Nrf2 (n = 5/gender) by the bDNA assay. Data are expressed as percent control. Values are
expressed as mean ± S.E.M.. Asterisks (*) indicate statistically significant differences between control and treatment groups ($p \leq 0.05$).

Figure 5. Messenger RNA expression of Ugt2a3 and Ugt3a1/2 in livers of male mice treated with three activators of AhR, CAR, PXR, PPARα, and Nrf2 ($n = 5$/gender) by the bDNA assay. Data are expressed as percent control. Values are expressed as mean ± S.E.M.. Asterisks (*) indicate statistically significant differences between control and treatment groups ($p \leq 0.05$).

Figure 6. Messenger RNA expression of Ugt1a1, 1a6, 1a7, 2a3, 2b34, and 2b35 in duodenum, jejunum, ileum, and colon of control and chemically treated mice by the bDNA assay. Treatments include prototypical activators TCDD, TCPOBOP, PCN, CLFB, and BHA for each tissue examined. Samples from $n=5$/group/treatment were pooled in equal amounts to perform the bDNA for each group in a single well. Data are expressed as Relative Light Units / 10 µg of pooled total RNA.

Figure 7. A) Messenger RNA expression of Ugt1a6 in duodenum, jejunum, ileum, and colon treated by corn oil or TCDD by the bDNA assay ($n = 5$/treatment). B) Messenger RNA expression of Ugt1a7 in duodenum, jejunum, ileum, and colon treated by corn oil or TCDD by the bDNA assay ($n = 5$/treatment). C) Messenger RNA expression of Ugt1a1, 1a6, 1a7, 2a3, 2b34, and 2b35 in duodenum of mice treated with corn oil or PCN. For parts A, B, and C, data are expressed as percent. Values are expressed as
mean ± S.E.M.. Asterisks (*) indicate statistically significant differences between control and treatment groups ($p \leq 0.05$).
Table 1.

List of prototypical microsomal enzyme inducers (MEIs) used to activate xenobiotic-responsive transcription factor pathways. Chemicals are sorted by prototypical pathways. Abbreviations, doses, vehicle, and route of administration are also listed.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Dose</th>
<th>Vehicle</th>
<th>Route</th>
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<tr>
<td><strong>AhR Activators</strong></td>
<td></td>
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</tr>
<tr>
<td>2,3,7,8-tetrachloro-dibenzodioxin (TCDD)</td>
<td>34 μg/kg</td>
<td>Corn Oil</td>
<td>IP</td>
</tr>
<tr>
<td>PCB 126</td>
<td>300 μg/kg</td>
<td>Corn Oil</td>
<td>IP</td>
</tr>
<tr>
<td>β-naphthaflavone (BNF)</td>
<td>200 mg/kg</td>
<td>Corn Oil</td>
<td>IP</td>
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<tr>
<td><strong>CAR Activators</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCPOBOP</td>
<td>300 μg/kg</td>
<td>Corn Oil</td>
<td>IP</td>
</tr>
<tr>
<td>Diallyl Sulfide (DAS)</td>
<td>200 mg/kg</td>
<td>Corn Oil</td>
<td>IP</td>
</tr>
<tr>
<td>Phenobarbital (PB)</td>
<td>100 mg/kg</td>
<td>Saline</td>
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<td><strong>PXR Activators</strong></td>
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<tr>
<td>Pregnenolone-16α-Carbonitrile (PCN)</td>
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<td>IP</td>
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<td>Spironolactone (SPR)</td>
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<td>Dexamethasone (DEX)</td>
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<td><strong>PPARα Activators</strong></td>
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</tr>
<tr>
<td>Clofibrate (CLFB)</td>
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<td>Ciprofibrate (CPFB)</td>
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<tr>
<td>Diethyl-hexylphthalate (DEHP)</td>
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<td>Oral</td>
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<tr>
<td><strong>Nrf2 Activators</strong></td>
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<tr>
<td>Oltipraz (OPZ)</td>
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<tr>
<td>Ethoxyquin (ETHOXYQ)</td>
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<td>Oral</td>
</tr>
<tr>
<td>Butylated Hydroxyanisole (BHA)</td>
<td>350 mg/kg</td>
<td>Corn Oil</td>
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</table>

*IP, Intraperitoneal*
Table 2. Summary of Mouse Ugt mRNA expression in liver by activators of AhR, CAR, PXR, PPARα, and Nrf2.

<table>
<thead>
<tr>
<th></th>
<th>AhR</th>
<th>CAR</th>
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<th>PPARα</th>
<th>Nrf2</th>
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<tr>
<td>Ugt1a1</td>
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<tr>
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<tr>
<td>Ugt1a5</td>
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<td>↑</td>
</tr>
<tr>
<td>Ugt1a6</td>
<td>↑</td>
<td>─</td>
<td>↑</td>
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<td>↑</td>
</tr>
<tr>
<td>Ugt1a7</td>
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<td>─</td>
<td>─</td>
<td>─</td>
<td>─</td>
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<tr>
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<tr>
<td>Ugt1a10</td>
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<td>─</td>
<td>─</td>
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<tr>
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<td>↓</td>
<td>─</td>
<td>─</td>
<td>─</td>
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<tr>
<td>Ugt2b1</td>
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<td>─</td>
<td>─</td>
<td>─</td>
<td>↓</td>
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<tr>
<td>Ugt2b35</td>
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<td>Ugt2b36</td>
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<tr>
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</table>

↑ Increased mRNA expression by two or more chemical activators
↓ Decreased mRNA expression by two or more chemical activators
─ Little or no change by chemical activators
Figure 1
Figure 2
Figure 3
Figure 4
Figure 5
Figure 6

Ugt1a1

Ugt1a6

Ugt1a7

Ugt2a3

Ugt2b34

Ugt2b35

Duodenum  Jejunum  Ileum  Colon

Ugt mRNA (RLU/10 μg Total RNA)

CON  TCDD  TCPOBOP  PCN  CLFB  BHA
Figure 7

A

B

C

Duodenum

Ugt mRNA (Percent Control)

Ugt1a6

Ugt1a7

Duod Jej Ileum Colon

Duod Jej Ileum Colon

Ugt1a1 Ugt1a6 Ugt1a7 Ugt2a3 Ugt2b34 Ugt2b35

CON TCDD

CON TCDD

CON PCN