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Evidence for triclosan-induced activation of human and rodent xenobiotic nuclear receptors ☆

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ABSTRACT

The bacteriostat triclosan (2,4,4′-trichloro-2′-hydroxydiphenylether) (TCS) decreases rat serum thyroxine via putative nuclear receptor (NR) interaction(s) and subsequent transcriptional up-regulation of hepatic catabolism and clearance. However, due to the evolutionary divergence of the constitutive androstane and pregnane-X receptors (CAR, PXR), TCS-mediated downstream effects may be species-dependent. To test the hypothesis that TCS activates xenobiotic NRs across species, cell-based NR reporter assays were employed to assess potential activation of rat, mouse, and human PXR, and rat, mouse, and three splice variants of human CAR. TCS activated hPXR, acted as an inverse agonist of hCAR1, and as a weak agonist of hCAR3. TCS failed to activate rPXR in full-length receptor reporter assays, and instead acted as a modest inverse agonist of rCAR. Consistent with the rat data, TCS also failed to activate mPXR and was a modest inverse agonist of mCAR. These data suggest that TCS may interact with multiple NRs, including hPXR, hCAR1, hCAR3, and rCAR in order to potentially affect hepatic catabolism. Overall these data support the conclusion that TCS may interact with NRs to regulate hepatic catabolism and downstream thyroid hormone homeostasis in both rat and human models, though perhaps by divergent mechanisms.

1. Introduction

Triclosan (2,4,4′-trichloro-2′-hydroxydiphenylether) (TCS) decreases thyroxine (T4) in juvenile rats (Crofton et al., 2007; Paul et al., 2010b; Zorrilla et al., 2009) as well as in pregnant, lactating, and neonatal rats following perinatal exposures (Paul et al., 2012, 2010a). Decreased maternal T4 during gestation leads to irreversible decreases in neurodevelopment and motor function in humans (Haddow et al., 1999; Pop et al., 2003, 1999), and neurological deficits and alterations in the cellular organization of the brain in rat models (Auso et al., 2004; Gilbert et al., 2000; Gilbert and Sui, 2006; Goldey and Crofton, 1998; Goldey et al., 1995; Lavado-Autric et al., 2003; Opazo et al., 2008). Though the general mechanisms for thyroid hormone maintenance and biology are conserved across species (Yen, 2001; Zoeller et al., 2007), interspecies-differences can present a major uncertainty when using solely *in vivo* laboratory animal data for predicting potential human health hazard.

Development of a proposed adverse outcome pathway (AOP) in rats enables parallel hypotheses to be investigated for plausibility in humans using *in vitro* models (Boobis et al., 2008; Capen, 1994; Hill et al., 1998). An AOP for TCS-induced hypothyroxinemia in rats has been proposed as increased hepatic catabolism of thyroid hormones (Crofton and Zoeller, 2005; Paul et al., 2012, 2010b), subsequent to interactions with the xenobiotic nuclear receptors, the constitutive androstane and pregnane-X receptors

Abbreviations: AOP, adverse outcome pathway; hCAR, human constitutive androstane receptor; mCAR, mouse constitutive androstane receptor; rCAR, rat constitutive androstane receptor; cDNA, complementary DNA; CITCO, 6-(4-chloro phenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde-O-(3,4-dichlorobenzyl) oxime; CTZ, clotrimazole; CYP, cytochrome P450; DEHP, di-(2-ethylhexyl)phthalate; DPX2, name of the hPXR model, commercial product of Puracyp, Inc; EC50, half-maximal effect concentration; LBD, ligand binding domain; MIE, molecular-initiating event; PB, phenobarbital; hPXR, human PXR; mPXR, mouse PXR; rPXR, rat PXR; RPXR, name of the rPXR model, commercial product of Puracyp, Inc; T4, thyroxine; TCPOBOP, 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene; TCS, triclosan.

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(CAR, PXR). CAR and PXR function at the nexus of exogenous and endogenous biology, regulating an overlapping set of genes necessary for euthyroid status (Kretschmer and Baldwin, 2005), including cytochrome P450s (CYPs), UDP-glucuronyltransferases (UGTs), sulfotransferases (SULTs), and drug transporters that mount metabolic responses to xenobiotics (Goodwin et al., 2001; Guo et al., 2003; Lehmann et al., 1998; Maglich et al., 2002; Moore et al., 2002; Wei et al., 2002; Zhou et al., 2005), and related genes that regulate hepatic energy metabolism (Konno et al., 2008; Maglich et al., 2009, 2003; Maglich et al., 2004). Generally, PXR and CAR are the primary transcriptional regulators of CYPs 2B and 3A (Wang and LeCluyse, 2003b). *In vivo* TCS exposure induced hepatic Cyp2b, Cyp3a, and Cyp4a protein and PROD and BROD activity in rats (Hanioka et al., 1997). *In vitro* TCS exposure increased Cyp2b and Cyp3a1 protein levels and enzymatic activity as well as markers of cytochrome P450 activity including pentoxyresorufin-O-depentylase (PROD), benzyloxyresorufin-O-debentylase (BROD), and ethoxyresorufin-O-deethylase (EROD) in rat hepatocytes (Jinno et al., 1997). Evidence for Cyp2b and Cyp3a induction *in vitro* and *in vivo*, and *in vitro* human pregnane-X receptor (PXR) activation by TCS (Jacobs et al., 2005), supports the hypothesis that TCS activates CAR and/or PXR, (Goodwin et al., 2001; Maglich et al., 2002; Moore et al., 2002).

Determination of the precise receptor isoforms that TCS may interact with is complex; the interaction may be species-dependent, and there is considerable cross-talk between receptors due to competition for an overlapping set of promoter response elements (Faucette et al., 2006; Goodwin et al., 2001; Istrate et al., 2010; Smirlis et al., 2001; Wei et al., 2002). Activation of human PXR (hPXR) results in transcriptional induction of CYP3A4 (Jones et al., 2000), Phase II hepatic biotransformation enzyme genes, i.e. those encoding UGTs and SULTs (Capen, 1994; DeVito et al., 1999; Schuur et al., 1997; Wang and James, 2006), and Phase III transporter genes, including those encoding organic anion transporting peptides and multidrug resistance proteins (Burk et al., 2005; Geick et al., 2001; Kretschmer and Baldwin, 2005) (Jansen et al., 2005). Similarly, activation of human CAR results in transcriptional up-regulation of *CYP2B6* (Maglich et al., 2002; Sueyoshi et al., 1999; Wang et al., 2004, 2003a), as well as *UGT1A1*, *SULT2A1*, and hepatic transporter genes including those encoding multi-drug resistance associated proteins MRP2 and MRP3 and the multi-drug resistance protein MDR1 (Urquhart et al., 2007). Murine knockout models have demonstrated that CAR and PXR are necessary for the downstream effects of phenobarbital (Qatani et al., 2005) and pregnenolone 16-carbontrile (PCN, (Chen et al., 2003; Cheng and Klaassen, 2006)) on glucuronidation and thyroid hormone elimination. Increased expression and activity of the UGT1a family of glucuronyltransferases that conjugate thyroid hormones (Barter and Klaassen, 1994; Liu et al., 1995) is a well-established mode-of-action (MOA) of thyroid hormone disruption for microsomal enzyme inducers that activate CAR and PXR (Buckley and Klaassen, 2009; Kretschmer and Baldwin, 2005). Activation of CAR and/or PXR is the putative molecular initiating event (MIE) that may result in augmented metabolic enzymes that catabolize and transport T4 following TCS exposure, potentially decreasing serum T4 concentrations.

However, activation of CAR and PXR by xenobiotics that are also known to perturb thyroid hormones is often species-dependent. For example, the compounds 6-(4-chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde-O-(3,4-dichlorobenzyl)oxime (CITCO), carbamazepine, efavirenz, and nevirapine preferentially activate human CAR and not mouse nor rat CAR (Chang and Waxman, 2006; Faucette et al., 2007; Moore et al., 2000). Conversely,1,4-bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP) is a selective murine CAR activator (Buckley and Klaassen, 2009; Maglich et al., 2009; Tzameli et al., 2000) and does not activate human CAR. Rifampicin and hyperforin selectively activate human PXR (Jones et al., 2000; LeCluyse, 2001; Lehmann et al., 1998; Sinz et al., 2007; Watkins et al., 2003), and PCN is a selective rodent PXR agonist (Cheng and Klaassen, 2006; Cheng et al., 2005; Guo et al., 2002a,b; Sinz et al., 2007). Some chemicals can activate both human and rodent receptors, including TO-901317 for PXR (Mitro et al., 2007) and di-(2-ethylhexyl) phthalate (DEHP) (DeKeyser et al., 2009; Ren et al., 2010) and phenobarbital for CAR (Mutoh et al., 2009; Qatanani and Moore, 2005a; Sueyoshi and Negishi, 2001; Yueh et al., 2005). Two contributing factors to this species-dependence phenomenon are: (1) the divergence of the ligand-binding domains within the receptors across species; and, (2) differential expression of splice variants of these receptors across species. PXR ligand-binding domains (LBDs) have diverged such that the LBD of rat and mouse PXR are 75–77% homologous to the LBD of human PXR (Jones et al., 2000; Moore et al., 2002). One significant difference between rodent CAR and human CAR is the variation in the transcriptome: rat CAR and mouse CAR have one isoform derived from a single mRNA expressed from their respective genes, while hCAR has at least three major isoforms derived from alternative splicing of the *NR1I3* mRNA. These three isoforms of human CAR are designated as hCAR1 or wildtype, hCAR2, and hCAR3 (Auerbach et al., 2003; Jinno et al., 2004; Savkur et al., 2003). The mRNA encoding the hCAR1 isoform comprises the majority of *NR1I3* mRNA expression in the liver. The hCAR2- and hCAR3-encoding splice variants together comprise up to 50% of the *NR1I3* transcriptome, and convey the majority of the hCAR ligand-binding activity (DeKeyser et al., 2009; Ross et al., 2010; Savkur et al., 2003). Given their implication in TCS-mediated hypothyroxinemia, these differences between rodent and human CAR and PXR highlight the need for a comparative, interspecies functional assessment of these receptors and their putative interactions with chemicals of environmental concern such as TCS.

Our previous work with TCS using a four-day exposure to young adult rats demonstrated that TCS exposure also increased markers of Phase II hepatic catabolism in addition to Phase I CYP activity and expression. Specifically, hepatic mRNA expression levels of *Cyp2b2*, *Cyp3a1/23*, *Ugt1a1*, and *Sult1c1*, as well as hepatic microsomal uridine diphosphate glucuronyltransferase (UGT) activity and pentoxyresorufin-O-deethylase (PROD) activity, were induced by TCS exposure (Paul et al., 2010b). These effects are consistent with other microsomal enzyme inducers that are thought to decrease thyroid hormones at least partially via up-regulation of hepatic catabolism (Barter and Klaassen, 1994; Chen et al., 2003; Hood et al., 2003; Vansell and Klaassen, 2002, 2001). In aggregate, the data suggest that TCS may activate CAR and/or PXR in the rat. The present work tested the hypothesis that TCS activates mouse, rat, and human PXR and/or CAR.

2. Materials and methods

2.1. Chemicals

Triclosan (⩾99%) was a gift from BASF/Ciba Specialty Chemicals. Control compounds for experiments, including rifampicing (RIF), clotrimazole (CTZ), CITCO, di-(2-ethylhexyl) phthalate (DEHP), dexamethasone, phenobarbital (PB), TO901317, and TCPOBOP were also obtained from Sigma (St. Louis, MO). Dimethyl sulfoxide (DMSO) (Sigma, ⩾99.7%) was the vehicle solvent for all test chemicals used. For experiments with the DPX2 and RPXR cell models (Puracyp, Inc.), chemicals were administered to 96-well plates of cells using a BioMek 2000 (Beckman Coulter; Brea, CA), which robotically transferred 327 nanoliters of chemical stock solution to each treated well. For experiments with CAR, chimeric mouse and human PXR models, and confirmation of the RPXR

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