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Aryl Hydrocarbon Receptor Activity of Tryptophan Metabolites in Young Adult Mouse Colonocytes[§]

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ABSTRACT

The tryptophan microbiota metabolites indole-3-acetate, indole-3-aldehyde, indole, and tryptamine are aryl hydrocarbon receptor (AhR) ligands, and in this study we investigated their AhR agonist and antagonist activities in nontransformed young adult mouse colonocyte (YAMC) cells. Using *Cyp1a1* mRNA as an Ah-responsive end point, we observed that the tryptophan metabolites were weak AhR agonists and partial antagonists in YAMC cells, and the pattern of activity was different from that previously observed in CaCo2 colon cancer cells. However, expansion of the end points to other Ah-responsive genes

including the *Cyp1b1*, the AhR repressor (*Ahrr*), and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-inducible poly(ADP-ribose) polymerase (*TiParp*) revealed a highly complex pattern of AhR agonist/antagonist activities that were both ligand- and gene-dependent. For example, the magnitude of induction of *Cyp1b1* mRNA was similar for TCDD, tryptamine, and indole-3-acetate, whereas lower induction was observed for indole and indole-3-aldehyde was inactive. These results suggest that the tryptophan metabolites identified in microbiota are selective AhR modulators.

Introduction

The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that was initially identified as the intracellular protein that bound the environmental toxicant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), related halogenated aromatics, and polynuclear aromatic hydrocarbons (Nebert et al., 1972; Poland et al., 1976; Gu et al., 2000). The role of the AhR in mediating the biochemical and toxic responses induced by TCDD and related compounds has been confirmed in AhR knockout (*AhR*^{−/−}) mice which are resistant to the effects of TCDD (Nebert et al., 1972; Fernandez-Salguero et al., 1995; Schmidt et al., 1996; Mimura et al., 1997). Ligand-dependent activation of the AhR results in formation of a nuclear complex with the AhR nuclear translocator (Arnt) protein which binds *cis*-xenobiotic response elements (XREs) in the *Cyp1a1* and other target gene promoters (Poland et al., 1976; Hankinson, 1995; Whitlock, 1999). However, several nonclassic pathways have been discovered, and these include AhR interactions with other nuclear partners, binding to nonconsensus *cis*-promoter elements, and also responses that are associated with the extranuclear AhR (Blankenship and Matsumura,

1997; Kim et al., 2000; Singh et al., 2007; Vogel et al., 2007; Li and Matsumura, 2008; Dong and Matsumura, 2009; Denison et al., 2011; Jackson et al., 2014; Vogel et al., 2014).

Since the initial discovery that the AhR binds toxic polychlorinated and polynuclear aromatic hydrocarbons, it has subsequently been shown that the AhR also binds structurally and functionally diverse ligands, including health-promoting phytochemicals such as indole-3-carbinol, flavonoids and extracts from fruits and vegetables, and a growing list of pharmaceuticals including omeprazole and other benzimidazoles (Bjeldanes et al., 1991; Denison et al., 1998; Song et al., 2002; Jeuken et al., 2003; Henry et al., 2006; Hu et al., 2007; Safe et al., 2012). In addition, structurally diverse “endogenous” biochemicals have been identified as AhR ligands, and there is evidence that the tryptophan photoproduct 6-formylindolo[3,2-*b*]carbazole (FICZ) and kynurenine may function as an endogenous ligand for the AhR (Song et al., 2002; Oberg et al., 2005; Henry et al., 2006; Wincent et al., 2009; Opitz et al., 2011).

The development of *AhR*^{−/−} and tissue-specific AhR knockout mice has been instrumental in showing that this receptor plays an essential role in various tissues and is a critical regulator of inflammation, autoimmune and immune responses and is a potential drug target for treating multiple diseases including cancer (Kerkvliet, 2009; Stevens et al., 2009; Marshall and Kerkvliet, 2010; Busbee et al., 2013; Safe et al., 2013). For example, there is extensive evidence that the AhR and its agonists including AhR-active cruciferous

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ABBREVIATIONS: AhR, aryl hydrocarbon receptor; *Ahrr*, AhR repressor; Arnt, aryl hydrocarbon receptor nuclear translocator; CH223191, CH, 2-methyl-*N*-[2-methyl-4-[(2-methylphenyl)diazenyl]phenyl]pyrazole-3-carboxamide; ChIP, chromatin immunoprecipitation; FICZ, 6-formylindolo[3,2-*b*]carbazole; IL-22, interleukin-22; ILC22, interleukin-22-producing innate lymphoid cells; PCR, polymerase chain reaction; SAhRMs, selective aryl hydrocarbon receptor modulators; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; *TiParp*, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-inducible poly(ADP-ribose) polymerase; XRE, xenobiotic response element; YAMC, young adult mouse colonocyte.

TABLE 1
Primers used in quantitative real-time PCR

Name	Forward Primer	Reverse Primer
TBP	GAACAATCCAGACTAGCAGCA	GGGAACCTTCACATCACAGCTC
Cyp1a1	CTGAAGTGGTCTCTGAGCGG	TCCACTCCATCTTCCGACTT
Cyp1b1	GGATATCAGCCACGACGAAT	ATTATCTGGGCAAAGCAACG
TiParp	GCCAGACTGTGTAGTACAGCC	GGGTTCCAGTTCCCAATCTTTT
Ahr	ACATACGCCGGTAGGAAGAGA	GGTCCAGCTCTGTATTGAGGC

vegetables play a protective role in mouse models of intestinal cancer and inflammation (Kawajiri et al., 2009; Arsenescu et al., 2011; Benson and Shepherd, 2011; Furumatsu et al., 2011; Kiss et al., 2011; Li et al., 2011; Monteleone et al., 2011; Singh et al., 2011; Lee et al., 2012).

Several studies have reported that the gut microbiota produces metabolites that include AhR-active compounds that could potentially modulate AhR-mediated intestinal resiliency and responses to inflammatory stimuli (Li et al., 2009; Bansal et al., 2010; Zelante et al., 2013; Fukumoto et al., 2014; Venkatesh et al., 2014). Research in our laboratories has previously investigated the AhR activities of the tryptophan metabolites indole, indole-3-acetate, tryptamine, and 3-indoxyl sulfate using CYP1A1 induction as a prototypical AhR-dependent response in human CaCo2 colon cancer cells (Jin et al., 2014). In this report, we determined the AhR activity of tryptophan metabolites in a nontransformed young adult mouse colonocyte (YAMC) cell line (D'Abaco et al., 1996), and there were significant differences between YAMC versus CaCo2 cells with respect to the gene-specific AhR agonist and antagonist activities of tryptophan metabolites.

Materials and Methods

Cell Lines, Antibodies, and Reagents. The YAMC cell line was initially generated from the Immorto mouse (Whitehead et al., 1993) and has been

previously used in our studies (Kolar et al., 2007; Weige et al., 2009; Turk et al., 2011). Cells were maintained in RPMI 1640 medium with 5% fetal bovine serum, 5 units/ml mouse interferon- γ (IF005) (EMD Millipore, Billerica, MA), 1% ITS “–” minus (insulin, transferrin, selenium) (41-400-045; Life Technologies, Grand Island, NY) at 33°C (permissive conditions). In preparation for experiments, cells were transferred to 37°C (nonpermissive conditions).

AhR antibody (BML-SA210) was purchased from Enzo Life Sciences (Farmingdale, NY). The assay for metabolic activity of the tryptophan metabolites was determined using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay essentially as described by Jin et al. (2014) (Supplemental Fig. 1). β -Actin (A1978) was purchased from Sigma-Aldrich (St. Louis, MO), and Cyp1a1 antibody was kindly provided by Dr. Paul Thomas (Rutgers University). Indole (>99%), indole-3-acetate (98%), indole-3-aldehyde (97%), and tryptamine (99%) were purchased from Sigma-Aldrich, and TCDD (99%) was synthesized in our laboratory. CH-223191 (2-methyl-N-[2-methyl-4-[(2-methylphenyl)diazenyl]phenyl]pyrazole-3-carboxamide) (cat. no. 3858) was purchased from Tocris Bioscience (Bristol, United Kingdom). GelRed Nucleic Acid Stain (RGB-4103) was purchased from Phenix Research Products (Candler, NC).

Chromatin Immunoprecipitation Assay. The chromatin immunoprecipitation (ChIP) assay was performed using the ChIP-IT Express Magnetic Chromatin Immunoprecipitation kit (Active Motif, Carlsbad, CA) according to the manufacturer's protocol. YAMC cells (1.2×10^7 cells) were treated with TCDD and/or compounds for 2 or 24 hours. The cells were then fixed with 1%

Fig. 1. Tryptophan metabolites and TCDD as inducers of *Cyp1a1* in YAMC cells. YAMC cells were treated for 24 hours with (A) tryptophan metabolites, (B) TCDD, (C) tryptophan metabolites plus CH, or (D) TCDD plus CH. Expression of *Cyp1a1* mRNA was determined by real-time PCR. Results are expressed as mean $\pm$ S.E. for three replicate determinations, and significant ($P < 0.05$) induction (*) (A and B) or inhibition by CH (**) (C and D) is indicated.

formaldehyde, and the cross-linking reaction was stopped by addition of 0.125 M glycine. After washing with phosphate-buffered saline, cells were scraped and pelleted. The collected cells were hypotonically lysed, and nuclei were collected and then sonicated to the desired chromatin length (200–1500 base pairs).

The sonicated chromatin was immunoprecipitated with normal rabbit IgG or AhR antibodies and protein A-conjugated magnetic beads at 4°C for overnight. After the magnetic beads were extensively washed, the protein-DNA crosslinks were reversed and eluted. DNA was prepared by proteinase K digestion followed by polymerase chain reaction (PCR) amplification. The *Cyp1a1* primers were 5'-AGG CTC TTC TCA CGC AAC TC-3' (sense) and 5'-CGG GTG CAG AGC TAT CTA AGT-3' (antisense); we then amplified a 207-base pair region of mouse *Cyp1a1* promoter, which contained the AhR-binding sequences. The PCR products were analyzed on a 2% agarose gel in the presence of GelRed Nucleic Acid Stain.

Quantitative Real-Time PCR. Total RNA was isolated using Zymo Quick RNA MiniPrep Kit (Zymo Research, Irvine, CA) according to the manufacturer's protocol. RNA was eluted with 35 μ l of RNase-free water and stored at -80°C. Real-time PCR was performed using iTaQ Universal SYBR Green One-Step Kit (Bio-Rad Laboratories, Hercules, CA). The primers used are shown in Table 1.

Western Blot Analysis. Cells (1×10^5) were plated in six-well plates in RPMI media containing 2.5% fetal bovine serum for 16 hours and then treated with different concentrations of the compounds for 24 hours. Cells were collected using high-salt buffer (50 mM HEPES, 0.5 mol/l NaCl, 1.5 mM MgCl₂, 1 mM EGTA, 10% glycerol, and 1% Triton-X-100) and 10 μ l/ml protease inhibitor cocktail (Sigma-Aldrich). Protein lysates were incubated for 5 minutes at 95°C before electrophoresis and then separated on 10% SDS-polyacrylamide gel electrophoresis 120 V for 2 to 3 hours. Proteins were transferred onto polyvinylidene difluoride membranes by wet electroblotting in a buffer containing 25 mM Tris, 192 mM glycine, and 20% methanol for 1.5 hours at 180 mA. Membranes were then blocked for 30 minutes with specific antibodies. Specific proteins were detected by the use of chemiluminescence and then were exposed to Kodak Image Station 4000-mm Pro (Carestream Health, Rochester, NY).

Statistical Analysis. Statistical significance of differences between the treatment groups was determined by an analysis of variance and/or Student's *t* test, and the levels of probability were noted. At least three repeated experiments were determined for each data point, and results are expressed as mean $\pm$ S.E.

Results

YAMC cells were treated with different concentrations of tryptamine (10–100 μ M), indole (50–500 μ M), indole-3-acetate (50–500 μ M), and indole-3-aldehyde (50–500 μ M), and the induction of *Cyp1a1* mRNA was determined (Fig. 1A). Tryptamine and indole-3-acetate significantly induced *Cyp1a1* mRNA levels (>10-fold) at concentrations of 50 and 500 μ M, respectively, whereas indole and indole-3-aldehyde were inactive.

In contrast 0.01–10 nM TCDD, the most potent AhR agonist, induced a >600-fold increase in *Cyp1a1* mRNA levels with maximal induction by 10 nM TCDD (Fig. 1B), as previously observed in CaCo2 cells (Jin et al., 2014). Induction of *Cyp1a1* mRNA by the tryptophan metabolites (Fig. 1C) and TCDD was inhibited after cotreatment with the AhR antagonist CH-223191 (CH) (Fig. 1D). In the inhibition experiment we observed some induction of *Cyp1a1* by indole and indole-3-aldehyde alone (Fig. 1C), and over several experiments low-level induction responses by these compounds were variable (0- to 4-fold) but <1% of the response observed for TCDD.

Previous studies in CaCo2 cells showed that indole was an AhR antagonist (Jin et al., 2014), and we further investigated the inhibitory effect of the tryptophan metabolites on induction of *Cyp1a1* by TCDD (Fig. 2A). All four compounds exhibited AhR antagonist activity, and both tryptamine and indole-3-aldehyde decreased induction of *Cyp1a1* mRNA by TCDD by >75%, which was more effective than observed for CH (Fig. 1D). Western blot analysis (Fig. 2B) showed that TCDD

Fig. 2. Tryptophan metabolites as AhR antagonists. YAMC cells were treated with tryptophan metabolites, TCDD, and their combination, and the effects on (A) *Cyp1a1* mRNA and (B) CYP1A1/AhR proteins were determined by real-time PCR and Western blot analysis. (C) YAMC cells were treated with 10 nM TCDD, 50 μ M tryptamine, or their combination for 2 and 24 hours and real-time PCR was used to determine interactions of the AhR with the *Cyp1a1* promoter (containing XRE) in a ChIP assay. (D) YAMC cells were treated with dimethylsulfoxide or 10 nM TCDD for 24 hours and also cotreated with 50 μ M tryptamine after 18, 20, 22, and 23 hours, and *Cyp1a1* mRNA was determined by real-time PCR. The results (A and D) are expressed as mean $\pm$ S.E. (three replicates), and significant ($P < 0.05$) inhibition is indicated (**).

but not the tryptophan metabolites decreased AhR protein expression, and in combination experiments AhR levels resembled that observed for TCDD alone.

TCDD induced Cyp1a1 protein in YAMC cells, whereas minimal induction was observed for the tryptophan metabolites and in combination experiments indole-3-acetate appeared to be the most effective inhibitor of TCDD-induced CYP1A1 protein. Unfortunately, CYP1A1 protein levels in YAMC cells were low, and the results of the TCDD + tryptophan metabolites studies were difficult to interpret.

We also examined the effects of TCDD, tryptamine, and their combination on recruitment of the AhR to the *Cyp1a1* XRE in a ChIP assay. After treatment of 2 hours, TCDD alone or in combination with tryptamine induced AhR interactions with the *Cyp1a1* promoter, whereas minimal effects were observed in YAMC cells treated with tryptamine alone (Fig. 2C). These results contrasted to those observed in CaCo2 cells where indole, the most effective AhR antagonist, blocked TCDD-induced AhR interactions with the ICyp1a1 promoter (Jin et al., 2014).

Analysis of these interactions were also investigated after treatment for 24 hours; significant AhR recruitment to the *Cyp1a1* promoter was observed after treatment with tryptamine alone, and a comparison of the results of 2- and 24-hour treatments suggested that the tryptamine-induced AhR recruitment was a relatively slow process. In contrast, after treatment with TCDD for 24 hours, the AhR binding to the *Cyp1a1* promoter was decreased, and this was consistent with the observed TCDD-induced degradation of the AhR protein (Fig. 2B).

Despite the inhibition of TCDD-induced *Cyp1a1* mRNA levels by 24 hours of tryptamine treatment (Fig. 2A), AhR binding to the *Cyp1a1* promoter in the combined treatment group was essentially additive (Fig. 2C). Therefore, it is possible that the inhibition of TCDD-induced *Cyp1a1* by tryptamine is posttranscriptional and AhR-independent.

YAMC cells were treated with TCDD alone for 24 hours and cotreated with tryptamine after 18, 20, 22 and 23 hours after addition of TCDD. The results showed that there was a time-dependent decrease in induced *Cyp1a1* mRNA (Fig. 2D), suggesting that some of the inhibitory effects of tryptamine were posttranscriptional and may be due to destabilization of *Cyp1a1* mRNA.

The tryptophan metabolites exhibited structure-dependent AhR agonist/antagonist activities with respect to induction of *Cyp1a1* in YAMC cells, and this pattern of activity was investigated with other Ah-responsive genes (Savas et al., 1994; D'Abaco et al., 1996; Baba et al., 2001; Diani-Moore et al., 2010). The results in Fig. 3A show that TCDD but not indole-3-aldehyde induced *Cyp1b1* expression in YAMC cells, and in combination studies indole-3-aldehyde partially inhibited TCDD-induced *Cyp1b1* expression. Indole was a partial agonist for induction of *Cyp1b1* but did not inhibit TCDD-induced *Cyp1b1* mRNA levels (Fig. 3B). Indole-3-acetate (Fig. 3C) and tryptamine (Fig. 3D) induced *Cyp1b1* mRNA levels similar to that of TCDD and did not inhibit induction by TCDD, indicating that both compounds were full AhR agonists for induction of *Cyp1b1*.

A similar approach was used to examine the AhR agonist/antagonist activities of the tryptophan metabolites with respect to regulation of *Ahrr* and *TiParp* gene expression. Indole-3-aldehyde minimally induced *Ahrr* (<2-fold) at the highest concentration (500 μ M); TCDD induced *Ahrr* (<7-fold) and in combination indole-3-aldehyde was a weak AhR antagonist (Fig. 4A). Indole (Fig. 4B), indole-3-acetate (Fig. 4C), and tryptamine (Fig. 4D) were partial agonists and induced *Ahrr*, and only tryptamine exhibited partial AhR antagonist activity. Indole-3-aldehyde, indole, and indole-3-acetate (Fig. 5, A–C) did not induce *TiParp* or inhibit induction of *TiParp* by TCDD,

Fig. 3. Induction of *Cyp1b1*. YAMC cells were treated with TCDD (alone), indole-3-aldehyde (A), indole (B), indole-3-acetate (C), and tryptamine (D) alone and in combination with TCDD for 24 hours, and *Cyp1b1* was determined by real-time PCR. Results are expressed as mean $\pm$ S.E. (three replicates), and significant ($P < 0.05$) induction (*) or inhibition (**) of TCDD-induced *Cyp1b1* is indicated.

Fig. 4. Induction of *Ahrr*. YAMC cells were treated with TCDD (alone), indole-3-aldehyde (A), indole (B), indole-3-acetate (C), or tryptamine (D) alone and in combination with TCDD for 24 hours, and *Cyp1b1* was determined by real-time PCR. Results are expressed as mean $\pm$ S.E. (three replicates), and significant ($P < 0.05$) induction (*) or inhibition (**) of TCDD-induced *Ahrr* is indicated.

whereas tryptamine (Fig. 5D) exhibited partial agonist/antagonist activity. Thus, the effects of the tryptophan metabolites as AhR agonist and antagonists were highly gene specific in YAMC cells, and these difference are summarized in Table 2.

Discussion

The AhR is expressed in the gastrointestinal tract, and studies in animal models demonstrate that this receptor and its ligands play an important role in gut health and response to stressors and disease (Kawajiri et al., 2009; Arsenescu et al., 2011; Benson and Shepherd, 2011; Furumatsu et al., 2011; Kiss et al., 2011; Li et al., 2011; Monteleone et al., 2011; Singh et al., 2011; Lee et al., 2012). Loss of the AhR results in formation of colon tumors at the cecum, and this is accompanied by increased expression of β -catenin in the small intestine, whereas wild-type AhR^{+/+} mice do not develop tumors or overexpress β -catenin (Kawajiri et al., 2009). Apc^{min/+} mice, which express a mutation in the Apc tumor suppressor gene, were crossed with AhR^{+/-} (heterozygote) mice, and the resulting Apc^{min/+}/AhR^{+/-} mice were more susceptible to cecal tumorigenesis (Kawajiri et al., 2009). However, AhR-active botanic compounds such as indole-3-carbinol and diindolylmethane (from cruciferous vegetables) significantly suppressed intestinal tumorigenesis in Apc^{min/+} and Apc^{min/+} AhR^{+/-} mice (Kawajiri et al., 2009).

Gut interleukin (IL)-22-producing innate lymphoid cells (ILC22) cells and postnatal lymphoid tissue-inducer-like subsets express the AhR which is essential for many of their functions. For example, the loss of the AhR results in decreased expression of ILC22 and decreased protection against bacterial infections (Lee et al., 2012). The

AhR agonist TCDD has been shown to induce Notch1, which is differentially required for the development of various ILC22 and lymphoid tissue-inducer-like cell subtypes.

The important functions of the AhR in maintaining intestinal function and health and protection against bacterial infections has been described in several reports showing that the AhR and its ligands also protect against intestinal damage/inflammation in experimental models of colitis and Crohn's disease (Arsenescu et al., 2011; Furumatsu et al., 2011; Monteleone et al., 2011; Singh et al., 2011). The severity of the effects of 2,4-trinitrobenzene sulfonic acid-induced colitis (which resembles Crohn's disease) in mice was significantly decreased by treatment with the AhR agonists FICZ (Lee et al., 2012) and TCDD (Benson and Shepherd, 2011), and this was accompanied by suppression of several markers of inflammation. The severity of dextran sodium sulfate-induced colitis in mice was also decreased by the AhR agonists β -naphthoflavone (Furumatsu et al., 2011), TCDD (Benson and Shepherd, 2011), and FICZ (Lee et al., 2012); in the latter study, the AhR antagonist 2-methyl-2H-pyrazole-3-carboxylic acid enhanced the severity of the colitis (Lee et al., 2012).

Previously, we investigated the tryptophan metabolites in CaCo2 human colon cancer cells and demonstrated their ligand-dependent AhR agonist and antagonist activities based primarily on modulation of *CYP1A1* gene expression (Jin et al., 2014). We also observed similar responses in nontransformed YAMC cells where the most active AhR agonists for induction of *Cyp1a1* were tryptamine and indole-3-acetate; however, the fold induction by both compounds was $<3\%$ of that observed for TCDD. In contrast, both tryptamine and indole-3-aldehyde were potent inhibitors of TCDD-induced *Cyp1a1* in YAMC cells, whereas tryptamine was primarily a full AhR agonist in

Fig. 5. Induction of *TiParp*. YAMC cells were treated with TCDD (alone), indole-3-aldehyde (A), indole (B), indole-3-acetate (C), or tryptamine (D) alone and in combination with TCDD for 24 hours, and *TiParp* was determined by real-time PCR. Results are expressed as mean $\pm$ S.E. (three replicates), and significant ($P < 0.05$) induction (*) or inhibition (**) of TCDD-induced *TiParp* is indicated.

CaCo2 cells using *CYP1A1* mRNA as an end point, demonstrating the importance of cell context.

We also investigated the AhR activity of the four tryptophan metabolites using three additional Ah-responsive genes, namely, *Ahrr*, *Cyp1b1*, and *TiParp*, and the results indicated that the AhR agonist and antagonist activities were both compound- and gene-specific (Table 2). For example, tryptamine was a weak AhR agonist and partial antagonist for *Cyp1a1* mRNA expression; however, examination of the ligand-dependent recruitment of the AhR complex to the *Cyp1a1* promoter (Fig. 2C) did not readily explain a mechanism for the activity of tryptamine as an AhR agonist (Fig. 2A).

In a separate experiment, we observed that treatment of YAMC cells with TCDD alone for 24 hours maximally induced *Cyp1a1* mRNA, which could then be significantly decreased by addition of tryptamine 18, 20, or 22 hours after treatment with TCDD, suggesting that some of the inhibitory effects of tryptamine on induced *Cyp1a1* mRNA may be posttranscriptional. In contrast, tryptamine and TCDD induced similar levels of *Cyp1b1* mRNA, and tryptamine did not affect TCDD-induced *Cyp1b1*, indicating that tryptamine was a full AhR agonist for this response.

The cell context- and gene-specific AhR agonist and antagonist activities of the tryptophan metabolites are not unique and have been observed for other AhR ligands including 6-methyl-1,3,8-trichlorobenzofuran, flavonoids, and pharmaceuticals (Astroff et al.,

1988; Lu et al., 1996; McDougal et al., 2001; Zhou and Gasiewicz, 2003; Jin et al., 2012; Safe et al., 2012). We also observed that the AhR antagonist CH inhibited *Cyp1a1* induction by TCDD and the tryptophan metabolites, indicating that CH inhibited induction of *Cyp1a1* by a diverse spectrum of AhR ligands, as previously reported elsewhere (Choi et al., 2012). Ongoing studies show that CH also antagonized induction of *Cyp1b1* by TCDD and the tryptophan metabolites; however, CH did not antagonize induction of *Ahrr* or *TiParp* by TCDD and tryptophan metabolites (data not shown), and this is currently being investigated.

TABLE 2

Effects of the tryptophan metabolites as AhR agonists and antagonists are highly gene specific in YAMC cells

Metabolite	<i>Cyp1a1</i>		<i>Cyp1b1</i>		<i>Ahrr</i>		<i>TiParp</i>	
	Ag	Ant	Ag	Ant	Ag	Ant	Ag	Ant
TCDD	+	—	+	—	+	—	+	—
Indole-3-aldehyde	— ^a	+	—	—	—	—	—	—
Indole	— ^a	+	+	—	+	—	—	—
Indole-3-acetate	+	+	+	—	+	—	—	—
Tryptamine	+	+	+	—	+	—	+	+

^a Weak agonist activity and somewhat variable.

In summary, our results show that for a limited set of Ah-responsive genes the AhR agonist and antagonist activities of the tryptophan metabolites are gene specific in nontransformed YAMC cells and are different from that previously observed in CaCo2 cancer cells (Jin et al., 2014). Differences in the AhR agonist and antagonist activities of the tryptophan metabolites are due not only to the transformed versus nontransformed phenotype of CaCo2 and YAMC cells but also to their different human versus mouse origins. These results suggest that indole-3-aldehyde, indole, indole-3-acetate, and tryptamine are selective AhR modulators (Safe et al., 2013; Murray et al., 2014) based on the results observed in this study.

Although indole-3-aldehyde exhibited minimal AhR agonist activity, a recent report indicated that AhR-dependent induction of IL-22 by indole-3-aldehyde plays a key role in microbiota-mediated protection from fungal infection and colitis (Zelante et al., 2013); however, indole-3-aldehyde did not induce IL-22 in YAMC cells (data not shown). Current studies are evaluating the contributions of AhR-active tryptophan metabolites in YAMC and other mouse- and human-derived cell lines to identify in vitro models that mimic in vivo effects of these compounds and identify relevant end points such as IL-22 induction that will predict the effects of AhR-active microbiota metabolites on gut health.

Authorship Contributions

Participated in research design: Cheng, Jin, Allred, Jayaraman, Chapkin, Safe.

Conducted experiments: Cheng, Jin, Allred, Safe.

Contributed new reagents or analytic tools: Cheng, Jin, Allred, Jayaraman, Chapkin, Safe.

Performed data analysis: Cheng, Jin, Safe.

Wrote or contributed to the writing of the manuscript: Cheng, Jin, Allred, Jayaraman, Chapkin, Safe.

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