Clinical Toxicology (2009) **47**, 632–642
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ISSN: 1556-3650 print / 1556-9519 online
DOI: 10.1080/15563650903140423

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REVIEW

Relevance of the aryl hydrocarbon receptor (AhR) for clinical toxicology

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Introduction. The aryl hydrocarbon receptor (AhR) is a cellular signaling molecule infamous for mediating the toxicity of dioxins and related compounds. *Aim.* The aim of this review is to provide a background of AhR and to examine critically its role in chemical toxicity, in physiological systems, and its interaction with drugs and other compounds. *Toxicity*. The AhR is essential for the toxicity of dioxins and related chemicals. The AhR mediates the exquisite sensitivity of animals to dioxins, where as little as 2 ng/kg/day can yield striking adverse effects. *Physiological role of AhR.* The wide variety of adverse effects of dioxin argues for an important role of the AhR in a variety of physiological systems. Recent investigations have highlighted the role of AhR in the development of the brain and vasculature. *Drugs and other chemical activators of AhR.* The development of AhR agonists during drug development programs is sometimes inadvertent, but sometimes the target of development, and is yet further confirmation of the likely importance of AhR signaling in constitutive physiology. The presence of AhR agonists in the diet such as indolo-(3,2-b)-carbazole and 3,3′-diindolylmethane (metabolized from indole 3-carbinol), flavonoids, and sulforaphane and of endogenous activators of this signaling system such as eicosanoids, indirubin, bilirubin, cAMP, and tryptophan are suggestive that AhR activation is a normal physiological process and that it is the persistent and high-level stimulation of AhR by dioxins that is responsible for toxicity. *Conclusions*. AhR-mediated toxicity and physiology are highly relevant to clinical toxicology and drug development.

Keywords Dioxin; TCDD; Aryl hydrocarbon receptor; Toxicity

Aryl hydrocarbon receptor: mechanisms of action

The aryl hydrocarbon receptor (AhR) was discovered as a result of a program to understand the basis of chemical carcinogenesis. While investigating the ability of the polycyclic aromatic hydrocarbon (PAH), 3-methylcholanthrene, to cause ulceration in mouse skin and induce cytochrome P450 (P450) 1A1 (CYP1A1), it was found that a single gene locus controlled the responsiveness of different mouse strains to polycyclic compounds.[1–3] This was designated the *Ah* (short for aryl hydrocarbon) locus. The *Ah* locus controlled the induction of cytochrome P450 by PAHs, and the induced cytochromes P450 then metabolized PAHs to genotoxic metabolites, thereby showing a key role for Ah in skin ulceration and carcinogenesis.

The next step in understanding the mechanism of action of the Ah receptor was the characterization of the exceptionally potent and stable chemical, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), as an inducer of P450 in the skin of mice. TCDD induced P450 with 10-fold greater potency in Ah-responsive mice compared to Ah-nonresponsive mice.[4] This insight led to the demonstration that TCDD bound specifically and avidly to a receptor (the AhR) in mouse liver cytosol,[5] that the receptor in Ah-responsive mice bound TCDD with higher affinity than the receptor from Ah-nonresponsive mice,[6] and that the AhR is in fact the product of the *Ah* locus.[7] After a Herculean struggle, sufficient AhR protein was purified to reveal an N-terminal sequence,[8] the essential prerequisite to clone the mouse[9,10] and human[11] AhR genes.

The structure of the mouse AhR is shown in Fig. 1A. The human AhR encodes a protein of 848 amino acids, of which 10 are cleaved from the N-terminus. Notable domains include an N-terminal basic helix–loop–helix domain, involved in binding to DNA, a C-terminal transcription activation domain, and two "PAS" repeats in the middle of the protein, which include the region of the molecule that binds to ligands. PAS proteins are a superfamily of conditional transcription factors containing a PAS sequence; the term is derived from the founding members of this superfamily, namely **P**er ("period," regulator of circadian rhythms), **A**rnt ("Ah receptor nuclear translocator"), and **S**im ("single-minded" regulator of midline cell differentiation). The AhR protein requires a complex folding pathway involving the chaperones, heat shock protein 90 (HSP90), and AhR-interacting

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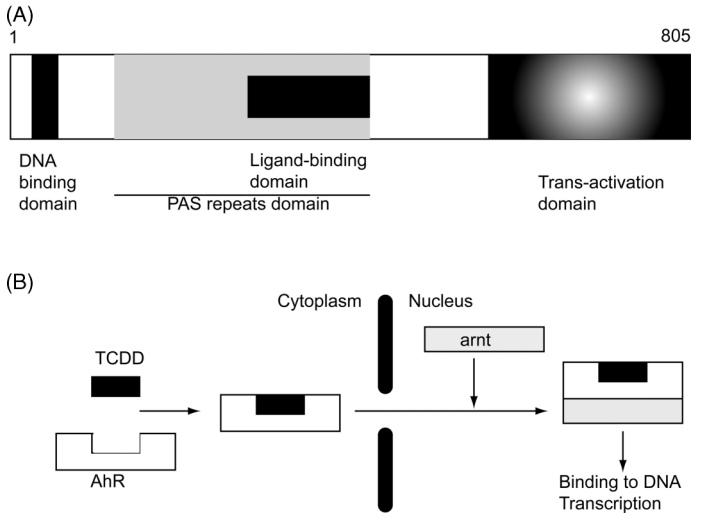

Fig. 1. (A) Cartoon of the primary structure of the Ah receptor. The mouse AhR^{b-1} receptor is shown as an 805 amino acid structure. The basic helix–loop–helix DNA-binding structure is shown as a black bar, and the two Per–Arnt–Sim (PAS) domain repeats are shown as a light gray box, enclosing a black box identifying the minimal residues necessary for ligand binding. The C-terminal transactivation domain is shown as a black- and gray-shaded region. (B) Schematic of AhR action. TCDD diffuses into the cell and binds to cytoplasmic AhR. The AhR then translocates into the nucleus, whereupon it binds to arnt. The AhR–arnt dimer then causes transcription of target genes.

protein (AIP, also known as XAP2 or ara9) among others[12] to achieve the functional ligand-binding form of the receptor. Removing HSP90[13] or AIP[14] from cells results in dysfunctional AhR, so it is known that these are required for correctly folding the AhR. However, it is not known with which chaperones the ligand-binding competent form of the AhR is associated directly.[15] This complex folding pathway results in low levels of expression of ligand-binding competent AhR,[16] which is a major difficulty in establishing the structural basis of ligand-binding to the AhR. The correctly folded AhR is located in the cytosol of cells with chaperone, until it binds to a ligand (Fig. 1B).

The AhR then translocates to the nucleus, dissociating from the chaperones, and forming a dimeric complex with another PAS family protein, arnt. The AhR–arnt complex then binds to particular sequences in DNA, known as xenobiotic response elements (XRE), and causes transcription of specific genes. The ligand-bound, active AhR is tightly regulated by the cell and is subject to rapid proteolytic degradation, so that >90% of nuclear AhR is exported from the nucleus and degraded within 4 h of treatment with ligand.[17] The addition of protein synthesis inhibitors prevents the TCDD-induced degradation of AhR, suggesting that a newly synthesized protein is required to degrade the AhR.[18]

Studies in AhR-null mice have confirmed that the AhR[19,20] and a functional DNA-binding domain in the AhR[21] are absolutely required for TCDD toxicity. It is known that specific alleles of the mouse AhR are determinative of the toxicity of dioxin,[22] and this is mediated via the affinity of the AhR for TCDD, with the AhR^b allele having ~10-fold greater avidity for TCDD compared to the AhR^d allele.[6] However, there is little evidence for any functional consequence of polymorphism in the AhR in humans despite extensive investigation.[23–25]

AhR ligands and toxicity

As suggested by the biochemical mechanism of action of AhR (Fig. 1B), the AhR[19,20] and a functional DNA-binding domain in the AhR[21] are required for TCDD toxicity. This provides conclusive evidence for the scheme of ligand-induced activation of the AhR, leading to transcription of specific target genes as the mechanism of toxicity. Thus, ligands of the AhR, which are agonists, are likely to have similar toxic effects.

Chloracne

Chloracne is a persistent eruption of comedones and epidermal cysts, typically around the eyes, behind the ears, and on the scrotum; an example is shown in Fig. 2. Chloracne was first associated with industrial production of chlorinated hydrocarbons in 1899,[27] and in 1937 polychlorinated naphthalenes and polychlorinated biphenyls were associated with three fatal cases of jaundice, one of whom had chloracne.[28] Industrial production of chlorinated hydrocarbons has given rise to a large number of poisonings, some of which are cited by Poland and Knutson.[29] Poisoning has occurred not only in an industrial setting but also following the contamination of rice with polychlorinated dibenzofurans in Yusho, Japan, and of rice-bran oil in Taiwan; each episode involved approximately 2,000 people.[30] Some aspects of chloracne have been reviewed recently.[31]

Humans show marked inter-individual variation in their response to TCDD, with chloracne occurring in some but not all poisoned individuals.[32] In those who develop chloracne, the minimum blood concentration of TCDD (or equivalents) was found to be approximately 1,100 pg TCDD/g of blood lipid.[33] A recent case report[26] was notable because of the high concentrations of TCDD (equivalent to a dose in excess of 1 mg) and the inter-individual response in chloracne, with an individual with 26 ng TCDD/g of blood lipids showing minimal chloracne (compared with <50 pg TCDD TEQ/g of blood lipid in unexposed humans). Chloracne is not restricted to dioxins and furans; other compounds have been shown to cause chloracne.[34,35]

TCDD toxicity

The toxicity of TCDD in animals presents as a florid collection of pathologies.[29,30] However, several features are notable.

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Fig. 2. Example of TCDD-induced chloracne. The photograph shows the ear of a patient at approximately 1 year after TCDD intoxication.[26] Photograph courtesy of A. Geusau, Medical University of Vienna.

First, the oral LD_{50} values vary over more than three orders of magnitude, with the guinea pig at ~1 μg/kg and the hamster in excess of 5,000 μg/kg. A marked difference in susceptibility is even possible in the same species and is known for both mice (the *AhR*^{b} and *AhR*^{d} loci[29]) and rats,[36] where susceptibility to acute lethality can vary by over 100-fold. Secondly, the toxic effects of TCDD are legion,[29] affecting a variety of organs and also causing carcinogenesis (through a nongenotoxic mechanism[37]) and teratogenesis. Recent studies in rats have demonstrated that TCDD is an extremely potent toxin during developmental exposure, with an acute dose of 1 μg/kg at gestational day 15 inducing ~25% lethality in the offspring,[38–40] or chronic exposure to 2 ng/kg/day inducing developmental delay.[41] There is little difference in the susceptibility of two rat strains to TCDD during development, whereas the same two strains show a ~100-fold difference in susceptibility to dioxin toxicity as adults.[42,43] Finally, it is remarkable how little is known about how TCDD causes toxic responses. Although it is known that the AhR is activated and gene transcription is required for toxicity, the identity of genes, or even pathways, that lead to toxicity is unclear. The possible exception to this is thyroid carcinogenesis in the rat, which is linked to hepatic induction of enzymes that metabolize thyroxine, subsequent feedback regulation of thyroid-stimulating hormone, and consequent trophic drive in the thyroid.

Toxicokinetics of TCDD

The toxicokinetics of TCDD deserve mention. TCDD has a half-life of approximately 2–3 weeks in rat,[44–46] although these pharmacokinetics are dose-dependent[47] as a result of hepatic induction of CYP1A2.[48] By contrast, the half-life of TCDD in human adults is approximately 7 years,[49] though this half-life is shorter in adults exposed to high levels of TCDD.[50] In human infants, the half-life is much shorter, with estimates ranging from 0.4 to 1.6 years.[51–53] The remarkable resistance to degradation and consequent long half-life of TCDD and related compounds are postulated to be a key factor in the toxicity of TCDD, as compared to more evanescent ligands of the AhR, such as methylcholanthrene.[54]

Endogenous function of AhR?

Although the many effects of TCDD and related chemicals make a clear case that activation of the AhR can affect physiological systems, these toxicity end points are not necessarily informative as to whether there is any endogenous role for the AhR. The clearest understanding of an endogenous role of the AhR comes from studies of the homologue in *Caenorhabditis elegans*, CeAhR. Although the CeAhR does not bind ligand[55] or the chaperone AIP,[56] it shows relatively high structural similarity to the mammalian AhR. Defects in CeAhR result in defects in neuronal migration, specific neural cell and axonal migration,[57] and cell fate.[58] The CeAhR also regulates behavior (aggregation) in *C. elegans*,[59] consistent with a role for this protein in regulating transcription. Although there is no direct evidence of a physiological role for the AhR in mammalian brain development or in behavior, there is evidence that the mouse AhR is activated in brain during normal development. A marker gene for AhR activation showed pronounced cell-specific and developmentally regulated activity in the brain,[60] suggesting that AhR is activated by a ligand during development in the brain and that the AhR has a role in brain development.

Although it is not proven that there is any physiological role of AhR in mammalian brain development, it is tantalizing that a mouse nullizygous for the three AhR-inducible *CYP1* genes showed severe developmental defects, including hydrocephalus.[61]

The *AhR* gene in mouse has been deleted ("knocked-out") by three separate laboratories.[62–64] Although about half of the AhR "knock-out" (*AhR*^{tm1Gonz}) mice initially died after birth,[62] the survivors were viable and fertile, as were their offspring, and the phenotype of neonatal lethality was lost. All three strains showed reduced weight gain and decreased

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liver weight,[62,63,65] which is now known to be due to defective development in the liver vasculature.[66] There is a failure to close the fetal liver ductus venosus in AhR-null mice.[67] This occurs as a result of malfunction of endothelial cells.[68] The role of AhR in vascular function is not restricted to the liver, as defects in eye and kidney vasculature are also present in AhR-null mice.[66] The classical scheme of a ligand binding to the AhR, and DNA binding and transcription, is informative for the developmental role of the AhR. Mice genetically engineered to have low levels of AhR show defects in liver vasculature development, and this phenotype is rescued by administration of a potent ligand (TCDD),[69] suggesting that normal development of the liver vasculature requires an AhR ligand. A mutation in the DNA-binding domain of the mouse AhR also leads to liver vasculature defects, showing that DNA binding and transcription is required for the developmental action of the AhR in the liver vasculature.[21]

Although the role of the AhR in liver biology is well described, understanding of its role in other systems awaits a full resolution. For example, there appears to be strong evidence that the AhR has a role in the immune system, in the TH_{17} cell,[70–72] but its precise role under physiological conditions remains unclear.[73]

Drugs and chemicals that interact with AhR

Drug–drug interactions

The AhR was originally discovered as a result of the ability of various chemicals and drugs to induce cytochrome P450 and hence alter the metabolism and pharmacokinetics of other drugs,[74] which has implications for clinical practice. Although omeprazole has been shown to be an inducer of cytochrome P450 CYP1A1,[75] and the induction of CYP1A may be of clinical relevance,[76] this is a comparatively high-dose effect. The ability of omeprazole to interact with other drugs has been studied[77] and the inhibitory effects of omeprazole on drug metabolism are more potent.[78]

Hu et al.[79] identified nine AhR agonists, six of which are marketed for human disease treatment. These include leflunomide (arthritis treatment), flutamide (prostate cancer treatment), and the calcium antagonist, nimodipine. Crucially, these drugs do not produce dioxin-like toxicity in rats or in humans, supporting the hypothesis that CYP1A1 induction and/or AhR activation is dependent upon the concentration of drug achieved and sustained *in vivo*. However, the potential for drug–drug interactions with AhR agonists is obvious and some examples are reviewed below.

Omeprazole

Omeprazole is a commonly prescribed drug used for treating gastro-esophageal reflux disease and duodenal ulcers. It induces CYP1A1 through AhR activation.[80] In human liver and hepatoma cells, AhR was shown to be involved in CYP1A1 and CYP1A2 induction by omeprazole. However, omeprazole's precise molecular mechanism has been a matter of controversy. In contrast to typical AhR ligands, such as 3-methylcholanthrene (3-MC) and β-naphthoflavone (β-NF), omeprazole appeared to activate AhR without direct binding. Indeed, Quattrochi and Tukey[81] demonstrated AhR activation but argued that induction of human CYP1A1 gene by omeprazole was AhR-independent.[82,83]

The claim that omeprazole activates AhR, but does so in the absence of binding to the AhR, is controversial, because of the wealth of data showing that ligand binding is essential for AhR activation. It is also experimentally challenging to distinguish low-affinity AhR binders from "nonbinders," and human AhR is particularly labile.[16] However, there is evidence that omeprazole is a low-affinity AhR ligand, competitively binding AhR with 300,000-fold weaker potency than TCDD.[84]

Unlike benzo[*a*]pyrene and TCDD, which are known to cause adverse effects, omeprazole has shown no experimental evidence of carcinogenic activity; indeed, benzo[*a*]pyrene cytotoxicity was inhibited by omeprazole exposure in a dose-dependent manner. It was concluded that the protective effect afforded by omeprazole against benzo[*a*]pyrene cytotoxicity depended upon the inhibition of CYP1A1.[80]

Drugs in development

Pifithrin-α

The tumor suppressor protein p53, a key regulator of DNA damage repair and apoptosis, is currently a target of emerging drug therapies directed toward neurodegenerative diseases such as Alzheimer's and Parkinson's. Of this group of drugs, the best characterized is pifithrin-α, a small molecule that inhibits p53-dependent apoptosis through an undetermined mechanism. Pifithrin-α is a potent AhR agonist as determined by its ability to bind AhR and upregulate marker genes such as CYP1A1. However, its ability to inhibit p53-mediated gene activation and apoptosis occurred in an AhR-independent manner. Hoagland et al.[85] hope to aid future design of more specific members of p53 inhibitors for clinical use.

Selective AhR modulators

TCDD

Activation of AhR by the xenobiotic ligand TCDD is hypothesized as the mechanism by which TCDD exerts its toxic and carcinogenic effects. TCDD exerts antiestrogenic responses, inhibiting 17β-estradiol-induced cell proliferation, progesterone receptor gene expression, and progesterone receptor binding. TCDD also inhibits spontaneous, age-dependent, and carcinogen-induced mammary tumor formation and growth in Sprague–Dawley rats, uterine cancer incidence,

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and MCF-7 xenograft growth in B6D2F$_1$-immunosuppressed mice.[86,87] Indeed, the accidental exposure of humans to TCDD in Seveso in 1976 led to decreased mammary and endometrial cancer incidence.[88,89] Such observations led to the hypothesis that selective AhR modulators (SAhRMs), AhR ligands that retain the antiestrogenic effects but lack the transcriptional effects of TCDD associated with toxicity, may be utilized as cancer chemotherapeutics in conjunction with other antiestrogenic compounds such as tamoxifen.

6-Methyl-1,3,8-trichlorodibenzo-furan and diindolylmethanes

6-Alkyl-1,3,8-trichlorodibenzofurans [e.g., 6-methyl-1,3,8-trichlorodibenzo-furan (6-MCDF)] and substituted diindolylmethanes (DIMs) represent two structural classes of SAhRMs. Studies suggest that substituted DIMs may possess therapeutic potential in the treatment of breast cancer.[90] 6-MCDF is a weak agonist and partial antagonist of AhR and also a partial estrogen receptor (ER) agonist.[91] SAhRMs are relatively nontoxic, inhibit mammary tumor growth, synergize with tamoxifen to inhibit breast cancer growth, and block tamoxifen-induced estrogenic activity in the uterus and thus represent a novel class of drugs for treatment of hormone-dependent cancers. Combined therapies of SAhRMs with tamoxifen and other selective ER modulators may provide a new approach for treating hormone-dependent cancer.[92]

Preliminary studies also indicate that SAhRMs inhibit prostate cancer cell growth, and there is evidence for inhibitory AhR–androgen receptor cross talk. Ftitz et al.[93] recently tested the hypothesis that 6-MCDF protects against prostate tumorigenesis. It had previously been suggested that AhR might possess tumor suppressor properties as ablation of AhR signaling increased susceptibility to prostate tumorigenesis. Prostate tumor incidence and size were not significantly reduced in mice fed with 6-MCDF. However, the frequency of pelvic lymph node metastasis was reduced fivefold in mice fed with 40 mg of 6-MCDF/kg. Serum vascular endothelial growth factor (VEGF) concentrations were also reduced by 6-MCDF treatment, particularly in mice without prostate tumors, and 6-MCDF was shown to act directly on cultured prostates to inhibit VEGF secretion. The results suggest that 6-MCDF may offer protection against prostate cancer progression, delaying metastasis, in part, by inhibiting VEGF production.

Antitumor aminophenylbenzothiazoles

The development of a series of potent and selective antitumor benzothiazoles from discovery of the lead compound, 2-(4-amino-3-methylphenyl)benzothiazole (DF 203), in 1995 to identification of the clinical candidate, Phortress®, now in Phase 1 clinical trial, is comprehensively reviewed by Bradshaw and Westwell.[94] The mechanism of action of this unique series of agents involves selective sequestration by sensitive cells,[95] followed by cytosolic AhR binding and translocation of AhR into the nucleus.[96] XRE-driven transcription of CYP1A1 precedes CYP1A1-catalyzed biotransformation of the drug to electrophilic reactive intermediate(s) that generate extensive DNA adducts resulting in tumor cell death.[97] Elucidation of this mechanism played a crucial role during development, specifically in synthesis of 2-(4-amino-3-methylphenyl)-5-fluorbenzothiazole (5F 203) to thwart CYP-catalyzed deactivating oxidative metabolism,[98] followed by water-soluble prodrug design to allow parenteral administration.[99]

The sensitivity of different tumor cell lines to these drugs has been shown to depend on AhR localization, with cytosolic localization (allowing drug–AhR binding) necessary for anti-tumor activity. Species differences in drug sensitivity also exist. For example, 5F 203 was a poor inducer of CYP1A1 in rat H4IIEC3 cells, but potent induction of CYP1A1 was observed in MCF-7 human mammary carcinoma cells.[100]

The evidence strongly suggests that AhR agonism and signal transduction are crucial to the antiproliferative activity of 5F 203. Moreover, aberrant AhR signaling may underlie resistance to aminophenylbenzothiazoles.

Aminoflavones

A similar class of novel antitumor agent, the aminoflavones, appear also critically dependent on AhR signaling. (5-Amino-2,3-fluorophenyl)-6,8-difluoro-7-methyl-4H-1-benzopyran-4-one (AF; NSC 686288) demonstrates differential antiproliferative activity in the NCI anticancer drug screen. MCF-7 human breast and papillary renal carcinoma cells are sensitive to aminoflavone both *in vitro* and when grown *in vivo* as xenografts in athymic mice.[101] Two US phase 1 dose escalation trials of the AF prodrug AFP464 are under way in patients with advanced solid tumors, specifically of the breast, kidney, and ovary (http://ClinicalTrials.gov).

AF activates AhR signaling, inducing its own metabolism by CYPs 1A1 and 1A2 and resulting in DNA–protein cross-links, phosphorylation of the histone H2AX (γ-H2AX), and apoptosis in sensitive tumor cells. In sensitive cells, AF treatment caused AhR translocation from the cytoplasm to the nucleus with subsequent formation of AhR–XRE protein DNA complexes.[101] In contrast, resistant cell lines demonstrated constitutive nuclear localization of AhR; therefore AF failed to induce CYP1A1 transcription, AhR–XRE complex formation, and apoptosis. In AF-sensitive, but not resistant, cells, 100-fold induction of CYP1A1 mRNA and a corresponding increase in ethoxyresorufin-*O*-deethylase activity were observed. These results suggest that the cytotoxicity of AF is the result of engagement of AhR-mediated signal transduction.[101]

Metabolism studies have shown that AF can be oxidized by CYP at two amino groups to form *N*-hydroxyl metabolites

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that are substrates for bioactivation by the sulfotransferase, SULT1A1. Meng et al.[102] propose that both *N*-sulfoxy groups can be further converted to nitrenium ions that form DNA and protein adducts. Inhibition of SULTS CYPs by natural flavonoids inhibited AF antiproliferative activity and diminished the formation of AF–DNA adducts. The results demonstrate the importance of AhR signal transduction, specifically CYP- and SULT1A1-metabolized AF activation for anticancer activity.[102] The mechanism of antitumor activity additionally involves formation of reactive oxygen species and oxidative DNA damage, as evidenced by increased 8-oxo-7,8-dihydroguanine. The antioxidant, acetylcysteine, and the CYP1A1/1A2 inhibitor, α-naphthoflavone, attenuated cytotoxic effects of AF.[103]

Dietary AhR agonists

Indolo-(3,2-b)-carbazole and 3,3′-DIM

The phytochemical indole 3-carbinol, a constituent of cruciferous vegetables, is metabolized within the body to generate the potent AhR agonist, indolo-(3,2-b)-carbazole,[104] and 3,3′-DIM), by acid-catalyzed dimerization of indole-3-carbinol in the stomach. Indole-3-carbinol in the maternal diet provides chemoprotection for the fetus against transplacental carcinogenesis by the PAH, dibenzo[a,l]pyrene.[105]

3,3′-DIM, a weak AhR agonist, significantly increased CYP1A1, CYP1B1, and CYP19 mRNA levels and ethoxyresorufin-O-deethylase activity. 3,3′-DIM inhibited carcinogen-induced mammary tumor growth in rodents and is associated with decreased breast cancer risk in humans. Such inhibition of estrogenic effects may arise via inhibitory AhR–ER cross talk,[106] ligand-activated AhR associated with the ER-α and the androgen receptor to promote hormone receptor proteolysis,[107] and modification of estrogen metabolism. By inducing the activity of CYP1A1 while suppressing that of CYP1B1, indole-3-carbinol is able to decrease production of carcinogenic 4-hydroxyestrone but increase CYP1A1-dependent 2-hydroxylation of estrogen. 2-Hydroxyestrone has been shown to possess chemoprotective properties.

Okino et al.[108] determined that the two AhR ligands were able to exert such fundamentally different effects through activation of distinct AhR-controlled pathways. Genetic knockdown of AhR confirmed that the effects of 3,3′-DIM and TCDD were indeed AhR-dependent. TCDD strongly induces AhR-dependent CYP1 gene expression, whereas 3,3′-DIM is a relatively weak cytochrome P450 inducer. 3,3′-DIM strongly inhibits ER-α expression and estrogen signaling, whereas TCDD has a notably weaker effect on these processes. In addition, indole-3-carbinol promotes apoptosis and, through perturbation of cyclin D, cell cycle inhibition. Such beneficial antitumor properties have fuelled interest in 3,3′-DIM analogs as putative anticancer therapeutic candidates.

Flavonoids

Flavonoids are present in fruits, vegetables, and beverages derived from plants (tea and red wine) and in many dietary supplements or herbal remedies, including Ginkgo Biloba, Soy, and Milk Thistle. Red clover isoflavones biochanin A and formononetin, used to ameliorate menopausal symptoms, are potent AhR ligands. These isoflavones are 10 times more potent than the indole compounds, indole-3-carbinol and 3,3′- DIM.[109]

Flavonoids have been postulated to be health-promoting, disease-preventing dietary supplements. Additionally, they have low toxicity, making them excellent candidates for chemoprevention. The cancer protective effects of flavonoids have been attributed to a wide variety of mechanisms, including agonism and antagonism of AhR, modulating metabolic enzyme activities, resulting in decreased carcinogenicity of xenobiotics. Moon et al.[110] have reviewed flavonoid effects on inhibition of CYP1A1, 1A2, 2E1, and 3A4 involved in the activation of procarcinogens and phase II enzymes, largely responsible for carcinogen detoxification.

Flavones (chrysin, baicalein, and galangin), flavanones (naringenin), and isoflavones (genistein, biochanin A) inhibit the activity of aromatase (CYP19), decreasing estrogen biosynthesis thus producing antiestrogenic effects, important in breast and prostate cancers. Activation of phase II detoxifying enzymes, such as UDP-glucuronyl transferase, glutathione S-transferase, and quinone reductase, by flavonoids results in carcinogen detoxification and represents one mechanism by which anticarcinogenic effects may be mediated. A number of flavonoids, including fisetin, galangin, quercetin, kaempferol, and genistein, are potent noncompetitive inhibitors of sulfotransferase 1A1 (SULT1A1); this may represent an important mechanism for the chemoprevention of sulfation-induced carcinogenesis.

Sulforaphane

Mechanisms underlying the chemoprotective properties of the isothiocyanate and sulforaphane, present in broccoli, revealed activation of AhR transformation and subsequent binding to an XRE. Sulforaphane induced CYP1A1 mRNA in a dose- and time-dependent manner, increasing transcription as early as 1 h in Hepa 1c1c7 and HepG2 cells. At the post-transcriptional level, sulforaphane did not affect the levels of existing CYP1A1 mRNA transcripts. Phase II drug-metabolizing enzymes were also induced. Thus broccoli-derived sulforaphane directly induces CYP1A1 gene expression in an AhR-dependent manner.[111]

Are there endogenous ligands?

The best known AhR ligands are exogenous compounds that released into the environment as, for example, products of imperfect combustion or contaminants of various herbicides

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and pesticides. However, the vital roles of AhR in diverse biological processes, such as vascular development and the immune system, suggest specific functions for endogenous ligands that modulate the activity of AhR. Until recently, the identity of such endogenous ligands has remained elusive.

Various classes of endogenous compounds, able to activate AhR, including tryptophan metabolites, indole-containing molecules, phenylethylamones,[112] bilirubin and biliverdin, sterols such as 7-ketocholesterol, horse steroid equilenin,[113] fatty acid metabolites including lipoxin A4 and prostaglandins, are considered below.

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Eicosanoids

Relatively high concentrations of several prostaglandins [PGB(3), PGD(3), PGF(3α), PGG(2), and PGH(2)] stimulate AhR transformation and DNA binding *in vitro*[114] inducing AhR-dependent reporter gene expression in mouse hepatoma cells in culture. PGG2 induced AhR-dependent reporter gene expression to a level three- to four-fold greater than observed with TCDD, and competitively displaced [^{3}H]–TCDD from AhR. It was concluded that selected prostaglandins are weak AhR agonists, representing a structurally distinct and novel class of AhR signal transduction activators.

Nebert and Karp[115] have argued that the myriad critical endogenous AhR functions mirror the universal actions of eicosanoids. Eicosanoids exert complex control over virtually all life functions. Interestingly, processes that exhibit abnormalities when AhR is absent or modulated by AhR activation are similar to eicosanoid-mediated functions. Fatty acid signaling molecules and metabolites, including arachidonic acid, prostaglandins, leukotrienes, and lipoxins, are widely metabolized by P450 isoforms, and eicosanoids represent a class of AhR ligands that induce CYP1A1 and their own metabolism. A product of arachidonic acid metabolism, lipoxin A4, possessing immunomodulatory and anti-inflammatory properties, is a potent endogenous AhR ligand mediating AhR transformation and DNA response element binding, driving transcription of CYP1A1. In competitive AhR-binding studies, lipoxin A4 displaced TCDD with an EC_{50} of 100 nM.

Indirubin

Zatloukalova et al.[116] compared the capacity of the endogenous ligand, indirubin, and TCDD to activate AhR signaling at several levels. Apart from similar effects of TCDD and indirubin on, for example, AhR-dependent cell cycle regulation, many differences were also observed, for example, in expression profile of AhR-target genes. The indirubin-induced AhR signaling significantly differs from AhR signaling induced by the exogenous ligand TCDD. Interestingly, indirubin is also a cyclin-dependent protein kinase inhibitor and may therefore impact cell cycle via AhR–cyclin-dependent protein kinase interactions. Clinically, indirubin has been used to treat chronic myelocytic leukemia.[117]

Bilirubin

The heme degradation product bilirubin acts as an AhR ligand and agonist.[84] The physiological relevance may be related to the ability of bilirubin to stimulate its own metabolism. TCDD is known to disrupt heme biosynthesis and enhance bilirubin degradation resulting in uroporphyria and hepatocellular damage. In congenitally jaundiced Gunn rats, persistent expression of CYP1A1 is observed, suggesting the presence of endogenous AhR ligands. Indeed, high bilirubin concentrations were detected, capable of inducing CYP1A1 in a dose- and AhR-dependent manner in cultured cells.

cAMP

The ubiquitous second messenger cAMP also represents a candidate AhR endogenous ligand.[118] However, given the concentration of these ligands in intact animals, concentrations required in cell culture and binding dissociation constants for such candidates are not as low as would be required to establish a physiological role for these AhR ligands.[115]

Tryptophan

Tryptophan has been shown to be converted to AhR agonists by UV light, or by aspartate aminotransferase to indole 3-pyruvate, which, in aqueous solution, spontaneously generates AhR agonists predominantly in heart tissue.[119] Tantalizing evidence[120] reveals that tryptophan-derived compounds, in particular 6-formylindolo[3,2-b]carbazole (FICZ), act as endogenous high-affinity AhR ligands.[121] Activation of AhR by UV radiation is mediated by the chromophoric amino acid tryptophan. Upon exposure to visible light, photolysis of tryptophan produces 6-formylindolo[3,2-b]carbazole, a substrate for CYPs 1A1, 1A2, and 1B1. Hydroxylated metabolites of 6-formylindolo[3,2-b]carbazole are substrates for sulfotransferases, SULT1A1, SULT 1A2, SULT1B1, and SULT1E1, and the sulfoconjugates of phenolic 6-formylindolo[3,2-b]carbazole metabolites are present in human urine. Wincent et al.[120] conclude that formylindolo[3,2-b]carbazoles represent potent endogenous activators of AhR signal transduction and key substrates of the CYP1 and SULT1 enzyme families.

Conclusions

Evidence is mounting to demonstrate the activation of AhR by endogenous ligands. Signal transduction networks are complex, and pathways may be activated in a transient and cell/tissue-specific manner to maintain homeostatic balance. As techniques become more sensitive, networks and molecules, which maintain such delicate homeostasis, may be further revealed, yielding the potential for modulating a variety of physiologically relevant pathways.

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Acknowledgments

D.R. Bell acknowledges A. Geusau (Medical University of Vienna) for permission to use the photograph, and Dow Chemical Company and the UK Food Standards Agency for grant support.

Declaration of interest: I have received funding for research on dioxins from the UK Food Standards Agency, and Dow Chemical Company, UK Central Science Laboratory.

References

1. Thomas PE, Kouri RE, Hutton JJ. Genetics of aryl hydrocarbon hydroxylase induction in mice – single gene difference between C57BL/6J and DBA/2J. Biochem Genet 1972; 6:157–168.
2. Nebert DW, Goujon FM, Gielen JE. Aryl hydrocarbon hydroxylase induction by polycyclic hydrocarbons – simple autosomal dominant trait in mouse. Nat New Biol 1972; 236:107–110.
3. Thomas PE, Hutton JJ, Taylor BA. Genetic relationship between aryl hydrocarbon hydroxylase inducibility and chemical carcinogen induced skin ulceration in mice. Genetics 1973; 74:655–659.
4. Poland A, Glover E. Genetic expression of arylhydrocarbon hydroxylase by 2,3,7,8-tetrachlorodibenzo-para-dioxin – evidence for a receptor mutation in genetically non-responsive mice. Mol Pharmacol 1975; 11:389–398.
5. Poland A, Glover E, Kende AS. Stereospecific, high affinity binding of 2,3,7,8-tetrachlorodibenzo-para-dioxin by hepatic cytosol – evidence that binding species is receptor for induction of arylhydrocarbon hydroxylase. J Biol Chem 1976; 251:4936–4946.
6. Poland A, Palen D, Glover E. Analysis of the 4 alleles of the murine arylhydrocarbon receptor. Mol Pharmacol 1994; 46:915–921.
7. Poland A, Glover E, Taylor BA. The murine Ah locus – a new allele and mapping to chromosome-12. Mol Pharmacol 1987; 32:471–478.
8. Bradfield CA, Glover E, Poland A. Purification and N-terminal amino-acid-sequence of the Ah receptor from the C57BL/6J mouse. Mol Pharmacol 1991; 39:13–19.
9. Burbach KM, Poland A, Bradfield CA. Cloning of the Ah-receptor cDNA reveals a distinctive ligand-activated transcription factor. Proc Natl Acad Sci USA 1992; 89:8185–8189.
10. Ema M, Sogawa K, Watanabe N, Chujoh Y, Matsushita N, Gotoh O, Funae Y, Fujii-Kuriyama Y. cDNA cloning and structure of mouse putative Ah receptor. Biochem Biophys Res Commun 1992; 184: 246–253.
11. Ema M, Matsushita N, Sogawa K, Ariyama T, Inazawa J, Nemoto T, Ota M, Oshimura M, Fujii-Kuriyama Y. Human aryl hydrocarbon receptor – functional expression and chromosomal assignment to 7p21. J Biochem 1994; 116:845–851.
12. Pratt WB, Toft DO. Regulation of signaling protein function and trafficking by the hsp90/hsp70-based chaperone machinery. Exp Biol Med 2003; 228:111–133.
13. Whitelaw ML, McGuire J, Picard D, Gustafsson JA, Poellinger L. Heat-shock protein Hsp90 regulates dioxin receptor function in-vivo. Proc Natl Acad Sci USA 1995; 92:4437–4441.
14. Lin BC, Nguyen LP, Walisser JA, Bradfield CA. A hypomorphic allele of aryl hydrocarbon receptor-associated protein-9 produces a phenocopy of the Ahr-null mouse. Mol Pharmacol 2008; 74:1367–1371.
15. Hollingshead BD, Petrulis JR, Perdew GH. The aryl hydrocarbon (Ah) receptor transcriptional regulator hepatitis B virus X-associated protein 2 antagonizes p23 binding to Ah receptor-Hsp90 complexes and is dispensable for receptor function. J Biol Chem 2004; 279:45652–45661.
16. Fan MQ, Bell AR, Bell DR, Clode S, Fernandes A, Foster PM, Fry JR, Jiang T, Loizou G, MacNicoll A, Miller BG, Rose M, Shaikh-Omar O, Tran L, White S. Recombinant expression of aryl hydrocarbon receptor for quantitative ligand-binding analysis. Anal Biochem 2009; 384:279–287.
17. Pollenz RS. The arylhydrocarbon receptor, but not the arylhydrocarbon receptor nuclear translocator protein, is rapidly depleted in hepatic and nonhepatic culture cells exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Mol Pharmacol 1996; 49:391–398.
18. Ma Q, Renzelli AJ, Baldwin KT, Antonini JM. Superinduction of CYP1A1 gene expression – regulation of 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced degradation of Ah receptor by cycloheximide. J Biol Chem 2000; 275:12676–12683.
19. Peters JM, Narotsky MG, Elizondo G, Fernandez-Salguero PM, Gonzalez FJ, Abbott BD. Amelioration of TCDD-induced teratogenesis in aryl hydrocarbon receptor (AhR)-null mice. Toxicol Sci 1999; 47:86–92.
20. Fernandez-Salguero PM, Hilbert DM, Rudikoff S, Ward JM, Gonzalez FJ. Arylhydrocarbon receptor-deficient mice are resistant to 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced toxicity. Toxicol Appl Pharmacol 1996; 140:173–179.
21. Bunger MK, Glover E, Moran SM, Walisser JA, Lahvis GP, Hsu EL, Bradfield CA. Abnormal liver development and resistance to 2,3,7,8-tetrachlorodibenzo-p-dioxin toxicity in mice carrying a mutation in the DNA-binding domain of the aryl hydrocarbon receptor. Toxicol Sci 2008; 106:83–92.
22. Nebert DW, Dalton TP, Okey AB, Gonzalez FJ. Role of aryl hydrocarbon receptor-mediated induction of the CYP1 enzymes in environmental toxicity and cancer. J Biol Chem 2004; 279:23847–23850.
23. Smart J, Daly AK. Variation in induced CYP1A1 levels: relationship to CYP1A1, Ah receptor and GSTM1 polymorphisms. Pharmacogenetics 2000; 10:11–24.
24. Okey AB, Boutros PC, Harper PA. Polymorphisms of human nuclear receptors that control expression of drug-metabolizing enzymes. Pharmacogenet Genomics 2005; 15:371–379.
25. Harper PA, Wong JMY, Lam MSM, Okey AB. Polymorphisms in the human AH receptor. Chem Biol Interact 2002; 141:161–187.
26. Geusau A, Abraham K, Geissler K, Sator MO, Stingl G, Tschachler E. Severe 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) intoxication: clinical and laboratory effects. Environ Health Perspect 2001; 109:865–869.
27. Herxheimer K. Uber chlorakne. Munch Med Wochenschr 1899; 46:278.
28. Drinker CK, Warren MF, Bennett GA. The problem of possible systemic effects from certain chlorinated hydrocarbons. J Ind Hyg Toxicol 1937; 19:283–311.
29. Poland A, Knutson JC. 2,3,7,8-Tetrachlorodibenzo-para-dioxin and related halogenated aromatic-hydrocarbons – examination of the mechanism of toxicity. Annu Rev Pharmacol Toxicol 1982; 22:517–554.
30. IPCS, ed. Polychlorinated Dbinzo-Para-Dioxins and Dibenzofurans. Vammala, Finland: World Health Organisation; 1988.
31. Yamamoto O, Tokura Y. Photocontact dermatitis and chloracne: two major occupational and environmental skin diseases induced by different actions of halogenated chemicals. J Dermatol Sci 2003; 32:85–94.
32. Oliver RM. Toxic effects of 2,3,7,8-tetrachlorodibenzo 1,4-dioxin in laboratory workers. Br J Ind Med 1975; 32:49–53.
33. Coenraads PJ, Olie K, Tang NJ. Blood lipid concentrations of dioxins and dibenzofurans causing chloracne. Br J Dermatol 1999; 141: 694–697.
34. Scerri L, Zaki I, Millard LG. Severe halogen acne due to a trifluoromethylpyrazole derivative and its resistance to isotretinoin. Br J Dermatol 1995; 132:144–148.
35. MacKenzie AR, Brooks S. Chloracnegen caution. Chem Ind 1998: 902, 912.
36. Pohjanvirta R, Tuomisto J. Han Wistar rats are exceptionally resistant to TCDD. 2. Arch Toxicol 1987; 11:344–347.
37. Poland A, Glover E. Estimate of the maximum in vivo covalent binding of 2,3,7,8-tetrachlorodibenzo-para-dioxin to rat-liver protein, ribosomal-RNA, and DNA. Cancer Res 1979; 39:3341–3344.
38. Bell DR, Clode S, Fan MQ, Fernandes A, Foster PM, Jiang T, Loizou G, MacNicoll A, Miller BG, Rose M, Tran L, White S. Toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the developing male Wistar

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(Han) rat. I: no decrease in epididymal sperm count after a single acute dose. Toxicol Sci 2007; 99:214–223.
39. Gray LE, Ostby JS, Kelce WR. A dose-response analysis of the reproductive effects of a single gestational dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin in male Long Evans Hooded rat offspring. Toxicol Appl Pharmacol 1997; 146:11–20.
40. Roman BL, Peterson RE. In utero and lactational exposure of the male rat to 2,3,7,8-tetrachlorodibenzo-p-dioxin impairs prostate development – 1. Effects on gene expression. Toxicol Appl Pharmacol 1998; 150:240–253.
41. Bell DR, Clode S, Fan MQ, Fernandes A, Foster PM, Jiang T, Loizou G, MacNicoll A, Miller BG, Rose M, Tran L, White S. Toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the developing male Wistar (Han) rat. II: chronic dosing causes developmental delay. Toxicol Sci 2007; 99:224–233.
42. Jiang T, Bell DR, Clode S, Fan MQ, Fernandes A, Foster PM, Loizou G, MacNicoll A, Miller BG, Rose M, Tran L, White S. A truncation in the aryl hydrocarbon receptor of the CRL: WI(Han) rat does not affect the developmental toxicity of TCDD. Toxicol Sci 2009; 107:512–521.
43. Huuskonen H, Unkila M, Pohjanvirta R, Tuomisto J. Developmental toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the most TCDD-resistant and TCDD-susceptible rat strains. Toxicol Appl Pharmacol 1994; 124:174–180.
44. Fries GF, Marrow GS. Retention and excretion of 2,3,7,8-tetrachlorodibenzo-para-dioxin by rats. J Agric Food Chem 1975; 23:265–269.
45. Rose JQ, Ramsey JC, Wentzler TH, Hummel RA, Gehring PJ. Fate of 2,3,7,8-tetrachlorodibenzo-para-dioxin following single and repeated oral doses to rat. Toxicol Appl Pharmacol 1976; 36:209–226.
46. Weber LWD, Ernst SW, Stahl BU, Rozman K. Tissue distribution and toxicokinetics of 2,3,7,8-tetrachlorodibenzo-p-dioxin in rats after intravenous injection. Fundam Appl Toxicol 1993; 21:523–534.
47. Bell DR, Clode S, Fan MQ, Fernandes A, Foster PM, Jiang T, Loizou G, MacNicoll A, Miller BG, Rose M, Tran L, White S. Relationships between tissue levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), mRNAs, and toxicity in the developing male Wistar(Han) rat. Toxicol Sci 2007; 99:591–604.
48. Poland A, Teitelbaum P, Glover E. [I-125]2-iodo-3,7,8-trichlorodibenzo-p-dioxin-binding species in mouse-liver induced by agonists for the Ah receptor – characterization and identification. Mol Pharmacol 1989; 36:113–120.
49. Geyer HJ, Schramm KW, Feicht EA, Behechti A, Steinberg C, Brüggemann R, Poiger H, Henkelmann B, Kettrup A. Half-lives of tetra-, penta-, hexa-, hepta-, and octachlorodibenzo-p-dioxin in rats, monkeys, and humans – a critical review. Chemosphere 2002; 48:631–644.
50. Aylward LL, Brunet RC, Carrier G, Hays SM, Cushing CA, Needham LL, Patterson DG Jr, Gerthoux PM, Brambilla P, Mocarelli P. Concentration-dependent TCDD elimination kinetics in humans: toxicokinetic modeling for moderately to highly exposed adults from Seveso, Italy, and Vienna, Austria, and impact on dose estimates for the NIOSH cohort. J Expo Anal Environ Epidemiol 2005; 15:51–65.
51. Kreuzer PE, Csanady GA, Baur C, Kessler W, Päpke O, Greim H, Filser JG. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and congeners in infants. A toxicokinetic model of human lifetime body burden by TCDD with special emphasis on its uptake by nutrition. Arch Toxicol 1997; 71:383–400.
52. Kerger BD, Leung HW, Scott P, Paustenbach DJ, Needham LL, Patterson DG Jr, Gerthoux PM, Mocarelli P. Age- and concentration-dependent elimination half-life of 2,3,7,8-tetrachlorodibenzo-p-dioxin in Seveso children. Environ Health Perspect 2006; 114:1596–1602.
53. Leung HW, Kerger BD, Paustenbach DJ. Elimination half-lives of selected polychlorinated dibenzodioxins and dibenzofurans in breast-fed human infants. J Toxicol Environ Health A 2006; 69:437–443.
54. Poland A, Glover E. Comparison of 2,3,7,8-tetrachlorodibenzo-para-dioxin, a potent inducer of arylhydrocarbon hydroxylase, with 3-methylcholanthrene. Mol Pharmacol 1974; 10:349–359.
55. Powell-Coffman JA, Bradfield CA, Wood WB. Caenorhabditis elegans orthologs of the aryl hydrocarbon receptor and its heterodimerization partner the aryl hydrocarbon receptor nuclear translocator. Proc Natl Acad Sci USA 1998; 95:2844–2849.
56. Bell DR, Poland A. Binding of aryl hydrocarbon receptor (AhR) to AhR-interacting protein – the role of hsp90. J Biol Chem 2000; 275:36407–36414.
57. Qin HT, Powell-Coffman JA. The Caenorhabditis elegans aryl hydrocarbon receptor, AHR-1, regulates neuronal development. Dev Biol 2004; 270:64–75.
58. Huang X, Powell-Coffman JA, Jin YS. The AHR-1 aryl hydrocarbon receptor and its co-factor the AHA-1 aryl hydrocarbon receptor nuclear translocator specify GABAergic neuron cell fate in C-elegans. Development 2004; 131:819–828.
59. Qin HT, Zhai ZW, Powell-Coffman JA. The Caenorhabditis elegans AHR-1 transcription complex controls expression of soluble guanylate cyclase genes in the URX neurons and regulates aggregation behavior. Dev Biol 2006; 298:606–615.
60. Campbell SJ, Henderson CJ, Anthony DC, Davidson D, Clark AJ, Wolf CR. The murine Cyp1a1 gene is expressed in a restricted spatial and temporal pattern during embryonic development. J Biol Chem 2005; 280:5828–5835.
61. Dragin N, Shi ZQ, Madan R, Karp CL, Sartor MA, Chen C, Gonzalez FJ, Nebert DW. Phenotype of the Cyp1a1/1a2/1b1(-/-) triple-knockout mouse. Mol Pharmacol 2007; 73:1844–1856.
62. Fernandez-Salguero P, Pineau T, Hilbert DM, McPhail T, Lee SS, Kimura S, Nebert DW, Rudikoff S, Ward JM, Gonzalez FJ. Immune-system impairment and hepatic-fibrosis in mice lacking the dioxin-binding Ah receptor. Science 1995; 268:722–726.
63. Schmidt JV, Su GHT, Reddy JK, Simon MC, Bradfield CA. Characterization of a murine Ahr null allele: involvement of the Ah receptor in hepatic growth and development. Proc Natl Acad Sci USA 1996; 93:6731–6736.
64. Mimura J, Yamashita K, Nakamura K, Morita M, Takagi TN, Nakao K, Ema M, Sogawa K, Yasuda M, Katsuki M, Fujii-Kuriyama Y. Loss of teratogenic response to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in mice lacking the Ah (dioxin) receptor. Genes Cells 1997; 2:645–654.
65. Shimizu Y, Nakatsuru Y, Ichinose M, Takahashi Y, Kume H, Mimura J, Fujii-Kuriyama Y, Ishikawa T. Benzo[a]pyrene carcinogenicity is lost in mice lacking the aryl hydrocarbon receptor. Proc Natl Acad Sci USA 2000; 97:779–782.
66. Lahvis GP, Lindell SL, Thomas RS, McCuskey RS, Murphy C, Glover E, Bentz M, Southard J, Bradfield CA. Portosystemic shunting and persistent fetal vascular structures in aryl hydrocarbon receptor-deficient mice. Proc Natl Acad Sci USA 2000; 97:10442–10447.
67. Lahvis GP, Pyzalski RW, Glover E, Pitot HC, McElwee MK, Bradfield CA. The aryl hydrocarbon receptor is required for developmental closure of the ductus venosus in the neonatal mouse. Mol Pharmacol 2005; 67:714–720.
68. Walisser JA, Glover E, Pande K, Liss AL, Bradfield CA. Aryl hydrocarbon receptor-dependent liver development and hepatotoxicity are mediated by different cell types. Proc Natl Acad Sci USA 2005; 102:17858–17863.
69. Walisser JA, Bunger MK, Glover E, Bradfield CA. Gestational exposure of Ahr and Arnt hypomorphs to dioxin rescues vascular development. Proc Natl Acad Sci USA 2004; 101:16677–16682.
70. Kimura A, Naka T, Nohara K, Fujii-Kuriyama Y, Kishimoto T. Aryl hydrocarbon receptor regulates Stat1 activation and participates in the development of Th17 cells. Proc Natl Acad Sci USA 2008; 105:9721–9726.
71. Quintana FJ, Basso AS, Iglesias AH, Korn T, Farez MF, Bettelli E, Caccamo M, Oukka M, Weiner HL. Control of T-reg and TH17 cell differentiation by the aryl hydrocarbon receptor. Nature 2008; 453:65–71.
72. Veldhoen M, Hirota K, Westendorf AM, Buer J, Dumoutier L, Renauld JC, Stockinger B. The aryl hydrocarbon receptor links T(H)17-cell-mediated autoimmunity to environmental toxins. Nature 2008; 453:106–109.
73. Esser C. The immune phenotype of AhR null mouse mutants: not a simple mirror of xenobiotic receptor over-activation. Biochem Pharmacol 2009; 77:597–607.

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74. Graham MJ, Lake BG. Induction of Drug Metabolism: Species Differences and Toxicological Relevance. Guildford, England: Elsevier Ireland Ltd; 2008:184–191.
75. Lu C, Li AP. Species comparison in P450 induction: effects of dexamethasone, omeprazole, and rifampin on P450 isoforms 1A and 3A in primary cultured hepatocytes from man, Sprague-Dawley rat, minipig, and beagle dog. Chem Biol Interact 2001; 134:271–281.
76. Fang AF, Damle BD, LaBadie RR, Crownover PH, Hewlett D, Glue PW. Significant decrease in nelfinavir systemic exposure after omeprazole coadministration in healthy subjects. Pharmacotherapy 2008; 28:42–50.
77. Robinson M, Horn J. Clinical pharmacology of proton pump inhibitors – what the practising physician needs to know. Drugs 2003; 63:2739–2754.
78. Furuta S, Kamada E, Suzuki T, Sugimoto T, Kawabata Y, Shinozaki Y, Sano H. Inhibition of drug metabolism in human liver microsomes by nizatidine, cimetidine and omeprazole. Xenobiotica 2001; 31:1–10.
79. Hu WY, Sorrentino C, Denison MS, Kolaja K, Fielden MR. Induction of Cyp1a1 is a nonspecific biomarker of aryl hydrocarbon receptor activation: results of large scale screening of pharmaceuticals and toxicants in vivo and in vitro. Mol Pharmacol 2007; 71:1475–1486.
80. Shiizaki K, Ohsako S, Kawanishi M, Yagi T. Omeprazole alleviates benzo[a]pyrene cytotoxicity by inhibition of CYP1A1 activity in human and mouse hepatoma cells. Basic Clin Pharmacol Toxicol 2008; 103:468–475.
81. Quattrochi LC, Tukey RH. Nuclear uptake of the Ah (Dioxin) receptor in response to omeprazole – transcriptional activation of the human Cyp1a1 gene. Mol Pharmacol 1993; 43:504–508.
82. Lemaire G, Delescluse C, Pralavorio M, Ledirac N, Lesca P, Rahmani R. The role of protein tyrosine kinases in CYP1A1 induction by omeprazole and thiabendazole in rat hepatocytes. Life Sci 2004; 74: 2265–2278.
83. Yoshinari K, Ueda R, Kusano K, Yoshimura T, Nagata K, Yamazoe Y. Omeprazole transactivates human CYP1A1 and CYP1A2 expression through the common regulatory region containing multiple xenobiotic-responsive elements. Biochem Pharmacol 2008; 76:139–145.
84. Denison MS, Nagy SR. Activation of the aryl hydrocarbon receptor by structurally diverse exogenous and endogenous chemicals. Annu Rev Pharmacol Toxicol 2003; 43:309–334.
85. Hoagland MS, Hoagland EM, Swanson HI. The p53 inhibitor pifithrin-alpha is a potent agonist of the aryl hydrocarbon receptor. J Pharmacol Exp Ther 2005; 314:603–610.
86. Kociba RJ, Keyes DG, Beyer JE, Carreon RM, Wade CE, Dittenber DA, Kalnins RP, Frauson LE, Park CN, Barnard SD, Hummel RA, Humiston CG. Results of a 2-year chronic toxicity and oncogenicity study of 2,3,7,8-tetrachlorodibenzo-para-dioxin in rats. Toxicol Appl Pharmacol 1978; 46:279–303.
87. Gierthy JF, Bennett JA, Bradley LM, Cutler DS. Correlation of in-vitro and in-vivo growth suppression of Mcf-7 human breast-cancer by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Cancer Res 1993; 53:3149–3153.
88. Bertazzi PA, Bernucci I, Brambilla G, Consonni D, Pesatori AC. The seveso studies on early and long-term effects of dioxin exposure: a review. Environ Health Perspect 1998; 106:625–633.
89. Bertazzi PA, Pesatori AC, Consonni D, Tironi A, Landi MT, Zocchetti C. Cancer incidence in a population accidentally exposed to 2,3,7,8-tetrachlorodibenzo-para-dioxin. Epidemiology 1993; 4:398–406.
90. McDougal A, Gupta MS, Morrow D, Ramamoorthy K, Lee JE, Safe SH. Methyl-substituted diindolylmethanes as inhibitors of estrogen-induced growth of T47D cells and mammary tumors in rats. Breast Cancer Res Treat 2001; 66:147–157.
91. Pearce ST, Liu H, Radhakrishnan I, Abdelrahim M, Safe S, Jordan VC. Interaction of the aryl hydrocarbon receptor ligand 6-methyl-1,3,8-trichlorodibenzofuran with estrogen receptor alpha. Cancer Res 2004; 64:2889–2897.
92. Safe S, Wormke M, Walker K, Dickerson R, Castro-Rivera E. Estrogen receptor signaling and crosstalk with the Ah receptor in endometrial cancer cells. In: Kuramoto H, Nishida M, eds. Cell and Molecular Biology of Endometrial Carcinoma. Tokyo, Japan: Springer-Verlag Tokyo; 2002:109–122.
93. Ftitz WA, Lin TM, Safe S, Moore RW, Peterson RE. The selective aryl hydrocarbon receptor modulator 6-methyl-1,3,8-trichlorodibenzofuran inhibits prostate tumor metastasis in TRAMP mice. Biochem Pharmacol 2009; 77:1151–1160.
94. Bradshaw TD, Westwell AD. The development of the antitumour benzothiazole prodrug, Phortress, as a clinical candidate. Curr Med Chem 2004; 11:1009–1021.
95. Chua MS, Kashiyama E, Bradshaw TD, Stinson SF, Brantley E, Sausville EA, Stevens MF. Role of CYP1A1 in modulation of antitumor properties of the novel agent 2-(4-amino-3-methylphenyl)benzothiazole (DF 203, NSC 674495) in human breast cancer cells. Cancer Res 2000; 60:5196–5203.
96. Loaiza-Perez AI, Trapani V, Hose C, Singh SS, Trepel JB, Stevens MF, Bradshaw TD, Sausville EA. Aryl hydrocarbon receptor mediates sensitivity of MCF-7 breast cancer cells to antitumor agent 2-(4-amino-3-methylphenyl) benzothiazole. Mol Pharmacol 2002; 61:13–19.
97. Leong CO, Gaskell M, Martin EA, Heydon RT, Farmer PB, Bibby MC, Cooper PA, Double JA, Bradshaw TD, Stevens MF. Antitumour 2-(4-aminophenyl)benzothiazoles generate DNA adducts in sensitive tumour cells in vitro and in vivo. Br J Cancer 2003; 88:470–477.
98. Hutchinson I, Chua MS, Browne HL, Trapani V, Bradshaw TD, Westwell AD, Stevens MF. Antitumor benzothiazoles. 14. Synthesis and in vitro biological properties of fluorinated 2-(4-aminophenyl)benzothiazoles. J Med Chem 2001; 44:1446–1455.
99. Bradshaw TD, Bibby MC, Double JA, Fichtner I, Cooper PA, Alley MC, Donohue S, Stinson SF, Tomaszewjski JE, Sausville EA, Stevens MF. Preclinical evaluation of amino acid prodrugs of novel antitumor 2-(4-amino-3-methylphenyl)benzothiazoles. Mol Cancer Ther 2002; 1:239–246.
100. Bazzi R, Bradshaw TD, Rowlands JC, Stevens MFG, Bell DR. 2-(4-Amino-3-methylphenyl)-5-fluorobenzothiazole is a ligand and shows species-specific partial agonism of the aryl hydrocarbon receptor. Toxicol Appl Pharmacol 2009; 237:102–110.
101. Loaiza-Perez AI, Kenney S, Boswell J, Hollingshead M, Alley MC, Hose C, Ciolino HP, Yeh GC, Trepel JB, Vistica DT, Sausville EA. Aryl hydrocarbon receptor activation of an antitumor aminoflavone: basis of selective toxicity for MCF-7 breast tumor cells. Mol Cancer Ther 2004; 3:715–725.
102. Meng LH, Shankavaram U, Chen C, Agama K, Fu HQ, Gonzalez FJ, Weinstein J, Pommier Y. Activation of aminoflavone (NSC 686288) by a sulfotransferase is required for the antiproliferative effect of the drug and for induction of histone gamma-H2AX. Cancer Res 2006; 66:9656–9664.
103. McLean L, Soto U, Agama K, Francis J, Jimenez R, Pommier Y, Sowers L, Brantley E. Aminoflavone induces oxidative DNA damage and reactive oxidative species-mediated apoptosis in breast cancer cells. Internat J Cancer 2006; 122:1665–1674.
104. Denison MS, Han DH, Nagy SR, Zhao B, Baston DS, Hayashi A, Knockaert M, Meijer L. Indirubins as activators of the aryl hydrocarbon receptor signal transduction pathway. In: Meijer L, Guyard N, Skaltsounis L, Eisenbrand G, eds. Indirubin, the Red Shade of Indigo. Les Eyzies de Tayac, France: Life in Progress Editions; 2003:157–167.
105. Yu JL, Nestrick TJ, Allen R, Savage PE. Microcontaminants in pentachlorophenol synthesis. 1. New bioassay for microcontaminant quantification. Ind Eng Chem Res 2006; 45:5199–5204.
106. Sanderson JT, Slobbe L, Lansbergen GWA, Safe S, van den Berg M. 2,3,7,8-Tetrachlorodibenzo-p-dioxin and diindolylmethanes differentially induce cytochrome P450 1A1, 1B1, and 19 in H295R human adrenocortical carcinoma cells. Toxicol Sci 2001; 61:40–48.
107. Ohtake F, Fujii-Kuriyama Y, Kato S. AhR acts as an E3 ubiquitin ligase to modulate steroid receptor functions. Biochem Pharmacol 2009; 77:474–484.
108. Okino ST, Pookot D, Basak S, Dahiya R. Toxic and chemopreventive ligands preferentially activate distinct aryl hydrocarbon receptor path-

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ways: implications for cancer prevention. Cancer Prev Res 2009; 2:251–256.
109. Medjakovic S, Jungbauer A. Red clover isoflavones biochanin a and formononetin are potent ligands of the human aryl hydrocarbon receptor. J Steroid Biochem Mol Biol 2008; 108:171–177.
110. Moon YJ, Wang XD, Morris ME. Dietary flavonoids: effects on xenobiotic and carcinogen metabolism. Toxicol In Vitro 2006; 20:187–210.
111. Anwar-Mohamed A, El-Kadi AOS. Sulforaphane induces CYP1A1 mRNA, protein, and catalytic activity levels via an AhR-dependent pathway in murine hepatoma Hepa 1c1c7 and human HepG2 cells. Cancer Lett 2009; 275:93–101.
112. Gielen JE, Nebert DW. Aryl hydrocarbon hydroxylase induction in mammalian liver cell culture.1. Stimulation of enzyme activity in nonhepatic cells and in hepatic cells by phenobarbital, polycyclic hydrocarbons, and 2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane. J Biol Chem 1971; 246:5189–5198.
113. Jinno A, Maruyama Y, Ishizuka M, Kazusaka A, Nakamura A, Fujita S. Induction of cytochrome P450-1A by the equine estrogen equilenin, a new endogenous aryl hydrocarbon receptor ligand. J Steroid Biochem Mol Biol 2006; 98:48–55.
114. Seidel SD, Winters GM, Rogers WJ, Ziccardi MH, Li V, Keser B, Denison MS. Activation of the Ah receptor signaling pathway by prostaglandins. J Biochem Mol Toxicol 2001; 15:187–196.
115. Nebert DW, Karp CL. Endogenous functions of the aryl hydrocarbon receptor (AHR): intersection of cytochrome P450 1 (CYP1)-metabolized eicosanoids and AHR Biology. J Biol Chem 2008; 283:36061–36065.
116. Zatloukalova J, Svihalkova-Sindlerova L, Kozubik A, Krcmar P, Machala M, Vondracek J. Beta-naphthoflavone and 3′-methoxy-4′-nitroflavone exert ambiguous effects on Ah receptor-dependent cell proliferation and gene expression in rat liver "stem-like" cells. Biochem Pharmacol 2007; 73:1622–1634.
117. Marko D, Schatzle S, Friedel A, Genzlinger A, Zankl H, Meijer L, Eisenbrand G. Inhibition of cyclin-dependent kinase 1 (CDK1) by indirubin derivatives in human tumour cells. Br J Cancer 2001; 84:283–289.
118. Baroukiabi R, Coumoul X, Fernandez-Salgueroc PM. The aryl hydrocarbon receptor, more than a xenobiotic-interacting protein. FEBS Lett 2007; 581:3608–3615.
119. Bittinger MA, Nguyen LP, Bradfield CA. Aspartate aminotransferase generates proagonists of the aryl hydrocarbon receptor. Mol Pharmacol 2003; 64:550–556.
120. Wincent E, Amini N, Luecke S, Glatt H, Bergman J, Crescenzi C, Rannug A, Rannug U. The suggested physiologic aryl hydrocarbon receptor activator and cytochrome P4501 substrate 6-formylindolo[3,2-b]carbazole is present in humans. J Biol Chem 2009; 284:2690–2696.
121. Rannug A, Fritsche E. The aryl hydrocarbon receptor and light. Biol Chem 2006; 387:1149–1157.

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