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Beyond the aryl hydrocarbon receptor: Pathway interactions in the hepatotoxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and related compounds

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Abstract

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the prototypical ligand for a group of environmental halogenated aromatic hydrocarbon contaminants which elicit hepatotoxicity and other toxic responses through activation of the aryl hydrocarbon receptor (AhR). Despite the conservation of the AhR and its signaling pathway, TCDD-elicited differential gene expression networks are species-specific, consistent with differences in sensitivity and toxic responses between species. This review integrates gene expression studies with complementary phenotypic analyses (e.g., metabolomics, clinical biochemistry, and histopathology) to elucidate the pathways through which TCDD and related compounds cause hepatotoxicity beyond AhR activation. We propose that AhR-mediated toxicity is a collective response to the cumulative burden of metabolic reprogramming across multiple pathways. Consequently, nutrition, health status, and genetic background establish the basis for differences in sensitivity and predisposition to adverse outcomes between species, sub-populations, tissues, and cells.

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Aryl hydrocarbon receptor, Hepatotoxicity, Toxicogenomics, Metabolic reprogramming, Species-specific sensitivity.

1. Introduction

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the prototypical member of a class of persistent environmental halogenated aromatic hydrocarbon (HAH) contaminants that elicit a broad spectrum of biochemical and toxic effects in a species-, sex-, age-, tissue-, and cell-specific manner. These effects include immuno-, hepato-, cardio-, and dermal toxicity, tumor promotion, teratogenicity, modulation of cell proliferation and differentiation, alterations in endocrine homeostasis, wasting, and lethality [1,2]. In addition to TCDD, there are 6 other polychlorinated dibenzo-*p*-dioxins (PCDDs), 10 polychlorinated dibenzofurans (PCDFs), and 12 co-planar polychlorinated biphenyl (PCBs) congeners, as well as chloro-substituted dioxin-like naphthalenes and diphenyl ethers that may also elicit dioxin-like toxicity [3]. Brominated analogs of PCDDs, PCDFs, and PCBs elicit comparable dioxin-like activity with relative effect potencies within one order of magnitude [4]. Environmental exposure typically involves a complex mixture of these compounds, which are assumed to share a common mechanism of action. Structure–activity relationships, antagonist studies, and knock-out models provide compelling evidence that most, if not all, of the toxic effects elicited by TCDD and dioxin-like compounds are mediated by the aryl hydrocarbon receptor (AhR). The AhR is a basic helix-loop-helix Per-Arnt-Sim (bHLH-PAS) domain-containing ligand activated transcription factor that is functionally similar but structurally different from members of the nuclear receptor superfamily [5,6].

In the canonical pathway, ligand binding to cytosolic AhR causes dissociation of heat shock protein 90 (HSP90), AhR-interacting protein (AIP; also known as ARA9 or XAP2), and p23, followed by nuclear translocation and dimerization with the AhR nuclear translocator (ARNT) [2,7,8]. The heterodimer complex then binds to dioxin response elements (DREs; core sequence 5′-GCGTG-3′) and recruits transcriptional coregulators, leading to the differential expression of target genes [2,7–9]. However, an increasing number of reports describe AhR-mediated changes in gene expression independent of DREs [2,5,10–12]. Moreover, the structure of the ligand can influence which coregulators are recruited to the promoter, similar to the selective modulation of nuclear receptors [13].

As we approach ~50 years of dioxin research, it is well established that the toxicity of TCDD and related compounds is due to changes in gene expression, yet the pathways affected and mechanisms of toxicity beyond AhR activation remain poorly understood. More specifically, which primary AhR-mediated gene expression responses trigger toxicity? Which secondary responses are required and/or contribute to the development of toxicity? Which metabolic pathways are most affected, and how does AhR-mediated metabolic reprogramming

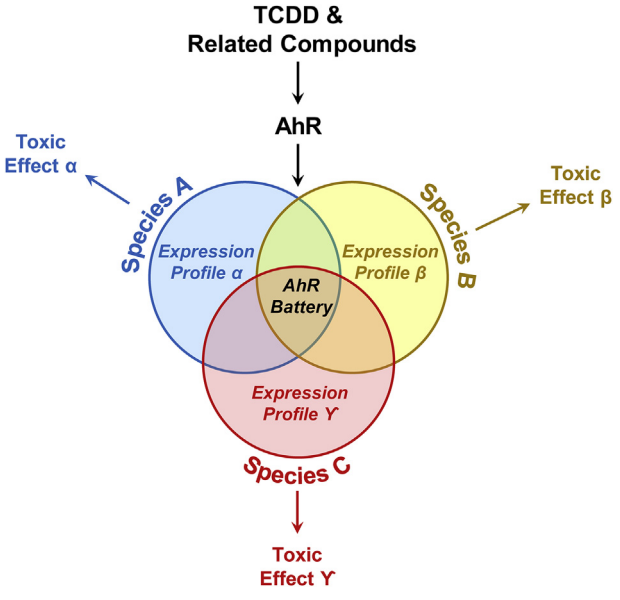
lead to adverse outcomes? Extending our mechanistic understanding of AhR-mediated toxicity is crucial not only to assess the relevance of rodent studies in predicting human risk, but also to identify vulnerable targets that are relevant to human diseases associated with metabolic disruption (e.g., diabetes, cardiovascular disease, metabolic syndrome) and potentially amenable to therapeutic intervention.

2. Comparative gene expression studies

Despite the conservation of the amino acid sequence, structural organization, and mode of action of the AhR, accumulating evidence suggests that AhR-mediated gene expression networks are not conserved across species, consistent with reported differences in species-specific adverse responses (Figure 1). Moreover, there is a 5000-fold difference in species-specific sensitivity to TCDD, with LD_{50} values ranging from 1 μg/kg for highly sensitive guinea pigs to 5000 μg/kg for resistant hamsters. Mice and rats are moderately sensitive with LD_{50} values of 114 and 22 μg/kg, respectively [14]. Using EC_{50} values for *Cyp1a1* induction as an indication of sensitivity, human primary hepatocytes are 2–50 times less sensitive to TCDD compared to rodent hepatocytes [15,16].

Computational analysis using a position weight matrix based on bona fide functional DREs revealed that DRE locations and distributions differ markedly between the human, mouse, and rat genomes [9]. Accordingly, transcriptomic analysis of TCDD-treated human, mouse, and rat primary hepatocytes identified only 16 orthologous genes that were differentially expressed in all three species, while the majority of orthologs exhibited species-specific expression [15]. Likewise, comparisons between human HepG2, mouse Hepa1c1c7, and rat H4IIE hepatoma cells report <8% of differentially expressed orthologs were conserved across all three species, with examples of divergent regulation (e.g., ortholog induced in one species but repressed in another) in the presence of conserved "AhR battery" gene responses (e.g., *Cyp1a1*, *Tiparp*, *Nqo1*, *Ugt1a6)* [17–19]. However, divergent gene expression between hepatoma cell lines and their relevance to human toxicity is rightly scrutinized due to model differences from inherent mutations, genetic instability, and clonal selection under differing culture conditions. Yet, *in vivo* studies also report minimal overlap (≤15%) between TCDD-responsive orthologs when comparing C57BL/6 mouse and Sprague–Dawley rat liver gene expression datasets [20,21]. Similarly, studies which integrate comparative gene expression analysis with complementary phenotypic measurements report species-specific changes in metabolites, serum biochemistry, and histopathology [16,22–25]. Although the AhR and its signaling pathway are highly conserved, these studies suggest AhR-mediated gene expression patterns are species-specific, which may account for differences in sensitivity between species.

Figure 1



Despite the conservation of the aryl hydrocarbon receptor and its signaling pathway, TCDD-elicited gene expression patterns are largely species-specific with minimal overlap between responsive orthologs (i.e., AhR gene battery). Species-specific target genes and the resulting toxic responses likely account for the reported differences in sensitivity and adverse effects between species.

3. Beyond gene expression – an integrated systems approach

3.1. Dysregulation of iron homeostasis and heme metabolism

In addition to facilitating comparative analyses, toxicogenomic studies can be used to further elucidate mechanisms of toxicity, which is particularly effective when integrated with complementary clinical biochemistry, metabolomics analysis, AhR enrichment (i.e., ChIP-chip, ChIP-Seq), and histopathology. This approach has been used to provide mechanistic insight into previously reported yet poorly understood adverse responses elicited by TCDD and related compounds. For example, Goldstein et al. (1973) identified a 60% increase in the hepatic iron levels of TCDD-treated mice [26]. Later, al-Turk et al. (1988) reported iron-supplementation in the diet increased TCDD-elicited lipid peroxidation while induced iron deficiency was protective [27]. More recently, duodenal epithelial and hepatic RNA-Seq analyses identified TCDD-elicited changes in gene expression consistent with iron overloading. Specifically, TCDD caused the dose-dependent repression of hepcidin, the master regulator of systemic iron homeostasis, resulting in a 2.6-fold increase in serum iron with accumulating levels spilling into urine [28]. Iron lies at the cross-section of multiple pathways