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Transcriptional profiling of rat white adipose tissue response to 2,3,7,8-tetrachlorodibenzo-ρ-dioxin

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ABSTRACT

Polychlorinated dibenzodioxins are environmental contaminants commonly produced as a by-product of industrial processes. The most potent of these, 2,3,7,8-tetrachlorodibenzo-ρ-dioxin (TCDD), is highly lipophilic, leading to bioaccumulation. White adipose tissue (WAT) is a major site for energy storage, and is one of the organs in which TCDD accumulates. In laboratory animals, exposure to TCDD causes numerous metabolic abnormalities, including a wasting syndrome. We therefore investigated the molecular effects of TCDD exposure on WAT by profiling the transcriptomic response of WAT to 100 μg/kg of TCDD at 1 or 4 days in TCDD-sensitive Long-Evans (*Turku/AB*; L-E) rats. A comparative analysis was conducted simultaneously in identically treated TCDD-resistant Han/Wistar (*Kuopio*; H/W) rats one day after exposure to the same dose. We sought to identify transcriptomic changes coinciding with the onset of toxicity, while gaining additional insight into later responses. More transcriptional responses to TCDD were observed at 4 days than at 1 day post-exposure, suggesting WAT shows mostly secondary responses. Two classic AHR-regulated genes, *Cyp1a1* and *Nqo1*, were significantly induced by TCDD in both strains, while several genes involved in the immune response, including *Ms4a7* and *F13a1* were altered in L-E rats alone. We compared genes affected by TCDD in rat WAT and human adipose cells, and observed little overlap. Interestingly, very few genes involved in lipid metabolism exhibited altered expression levels despite the pronounced lipid mobilization from peripheral fat pads by TCDD in L-E rats. Of these genes, the lipolysis-associated *Lpin1* was induced slightly over 2-fold in L-E rat WAT on day 4.

Background

2,3,7,8-tetrachlorodibenzo-ρ-dioxin (TCDD) is an organic toxicant introduced to the ecosystem as a by-product of industrial processes, such as low-temperature incineration of polyvinyl chlorides and pesticide production. A concerning biological property of TCDD lies in its highly stable and lipophilic nature. Once in the body, TCDD primarily localizes to liver and adipose tissues (Gasiewicz et al., 1983), resulting in an average half-life of 3 weeks in rats and 8 years in humans (Pohjanvirta et al., 1990; Geyer et al., 2002).

TCDD is a particularly potent ligand of the aryl hydrocarbon receptor (AHR) (Safe, 1990), a basic helix-loop-helix/PAS (bHLH/PAS) transcription factor highly conserved throughout evolution (Hahn et al., 1997). Normally bound to chaperone proteins situated in the cytosol, the AHR translocates to the nucleus upon ligand binding and activation, where it dimerizes with the AHR nuclear translocator (ARNT). The AHR/ARNT dimer binds to specific DNA response elements (AHRE-I and AHRE-II) and regulates transcription in a gene-specific manner (Dolwick et al., 1993; Denison and Whitlock, 1995; Sogawa et al., 2004). Substantial evidence indicates a primary role for the AHR in mediating TCDD toxicities: AHR-knockout mice (Fernandez-Salguero et al., 1996; Mimura et al., 1997), mice with ARNT-null hepatic tissue (Nukaya

Abbreviations: TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; WAT, white adipose tissue; L-E, Long-Evans rat; H/W, Han/Wistar rat; AHR, aryl hydrocarbon receptor.

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et al., 2010) or mice with AHRs that have a defective AHRE-binding domain (Bunger et al., 2003) all display significantly diminished phenotypic effects of TCDD insult relative to wild-type mice.

Exposure to TCDD elicits a wide range of toxicities that vary substantially in both nature and degree between (and even within) species. In humans, the hallmark of high TCDD exposure is a dermal condition known as chloracne, whereas laboratory animals demonstrate a diverse range of toxicological endpoints and sensitivities to toxic effects (Pohjanvirta et al., 1993; Kransler et al., 2007). One of the best-documented effects of TCDD exposure is a wasting syndrome characterized by rapid weight loss and subsequent lethality (Seefeld et al., 1984). This wasting syndrome is dose-dependent. Its severity can be reduced by a high-calorie diet, although lethality persists (Courtney et al., 1978). Although wasting syndrome occurs in most laboratory rodent species, not all animals are affected equally. A well-established model of inter-strain variation comprises the TCDD-sensitive Long-Evans (*Turku/AB*) rats (L-E; LD_{50} 9.8–17.7 μg/kg) and the TCDD-resistant Han/Wistar (*Kuopio*) rats (H/W; LD_{50} > 9600 μg/kg) (Pohjanvirta et al., 1993). The exceptional resistance of the H/W strain, especially to wasting syndrome and lethality, is attributed to a point mutation in the transactivation domain of the AHR (Pohjanvirta et al., 1998, 1999).

White adipose tissue (WAT) is potentially a very important interface between TCDD toxicokinetics and wasting syndrome physiology since WAT plays many roles: an endocrine organ involved in the regulation of food intake and energy metabolism (Ahima and Flier, 2000), an immune organ (Exley et al., 2014) and a major location of sequestered xenobiotics (Mullerova and Kopecky, 2007), including TCDD (Pohjanvirta et al., 1990). Considerable amounts of TCDD are shown to accumulate in WAT of both L-E and H/W as early as 1 day after exposure (Pohjanvirta et al., 1990), with a significant body weight loss ($p < 0.01$) observed 4 days following exposure in L-E but not in H/W (Lensu et al., 2011; Linden et al., 2014). Therefore, to investigate the role of WAT in TCDD-induced toxicities, particularly pertaining to wasting syndrome, we isolated WAT from dioxin-sensitive L-E rats and dioxin-resistant H/W rats 1 and 4 days following exposure to TCDD or vehicle control. WAT was additionally isolated from L-E rats treated with vehicle control and subjected to feed restriction for 4 days. The inclusion of feed-restricted animals allows for the differentiation of transcriptomic changes directly associated with exposure to TCDD from those resulting from secondary effects pertaining to the reduction in feed intake experienced by dioxin-sensitive rats. As a similar weight loss was not observed in H/W rats, this comparison was not replicated for this strain.

Materials and methods

Animal handling

Inbred, male, Long-Evans (L-E) and Han/Wistar (H/W) rats were obtained from the breeding colonies of the National Public Health Institute (Kuopio, Finland). Animals were housed individually in suspended stainless-steel, wire-mesh cages with pelleted R36 feed and tap water available *ad libitum*, with one exception: a subset of L-E rats was feed-restricted in which feed was reduced to amounts ingested by animals that had wasting syndrome (as described in (Pohjanvirta et al., 2008)). The housing environment was maintained at a temperature of 21 ± 1 °C and relative humidity of 50% ± 10%, with a 12 hour light/dark cycle. H/W rats were 15–16 weeks of age at time of treatment, while L-E rats were 18–19 weeks of age to ensure comparable body weights due to more rapid growth of H/W rats.

Experimental design

The experimental design is outlined in Fig. 1. Eight L-E and eight H/W rats were equally divided into treatment and control groups. Animals were treated by oral gavage with either 100 μg/kg of TCDD dissolved in corn oil or corn oil vehicle alone and euthanized 1 day after treatment. An additional cohort of twelve L-E rats was similarly divided into three groups, with two groups treated as above and the final group subjected to corn oil treatment accompanied by feed-restriction to mimic the reduced feed intake observed in TCDD-treated L-E rats (Pohjanvirta et al., 2008). This cohort was followed for 4 days, the point at which significant loss of body weight ($p < 0.01$) is observed in L-E but not H/W rats (Lensu et al., 2011; Linden et al., 2014). A similar experimental procedure has been described previously in studies of hepatic tissue (Linden et al., 2014). This treatment dose is essentially lethal to the TCDD-sensitive L-E rats, while being readily tolerated by the TCDD-resistant H/W strain. All animal treatment information is provided in Supplementary Table 1. At the end of the observation period, all animals were euthanized by decapitation; tissue was rapidly extracted and frozen in liquid nitrogen. All study plans were approved by the Animal Experiment Committee of the University of Kuopio and the Provincial Government of Eastern Finland. All animal handling and reporting comply with ARRIVE guidelines (Kilkenny et al., 2010).

Qiagen RNeasy kits were used to isolate total RNA from WAT according to the manufacturer's instructions (Qiagen, Mississauga, Canada). UV spectrophotometry was used to quantify total RNA yield and RNA integrity was verified using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). RNA was assayed on Affymetrix RAE230-2.0 arrays at The Centre for Applied Genomics at The Hospital for Sick Children (Toronto, Canada), following the standard protocols.

Statistical analysis

Raw data were loaded into the R statistical environment (v.3.1.2) using the affy package (v.1.44.0) of the BioConductor open-source project (Gautier et al., 2004; Gentleman et al., 2004). Probes were mapped and summarized using the custom rat2302rnentrezgcdf (v.19.0.0) package (Dai et al., 2005). Raw data were pre-processed using the RMA algorithm (Irizarry et al., 2003) and tested for spatial and distributional homogeneity (Supplementary Fig. 1). Unsupervised pattern recognition employed the complete linkage hierarchical clustering algorithm in the cluster package (v1.15.3) using Pearson's correlation as a similarity metric. The distribution of coefficients of variation for each experimental group was analyzed to quantify inter-replicate variation (Supplementary Fig. 2).

All statistical analyses were performed using the limma package (v3.22.1). Linear modeling was performed for each probeset to contrast TCDD-treated and control animals at each time-point (i.e. HWT–HWC and LET–LEC for the 24 h groups and LET–LER for the 96 h groups). An empirical Bayes method was used to reduce standard error amongst probes (Smyth, 2004) and moderated *t*-tests were used to compare each coefficient to zero. All experimental *p*-values were adjusted for multiple testing using a 5% false discovery rate (Storey and Tibshirani, 2003). Following *q*-value analysis, significance was defined as a *q*-value threshold <0.05. Results of the linear model are available in Supplementary Table 2. Raw and pre-processed data are available in the National Center for Biotechnology Information Gene Expression Omnibus (GEO ID: GSE18301).

Data visualization

Visualizations were generated using the lattice (v.0.20–29) and latticeExtra (v.0.6–26) packages. Normalized intensity values for the most variable probes across all samples (variance >0.1) were clustered using DIANA agglomerative hierarchical clustering, again using Pearson's correlation as a similarity metric. To visualize differences in tissue sensitivities following TCDD exposure, volcano plots were generated to compare results from multiple studies (Supplementary Fig. 3). Current array results from the 24 h time point were compared to results from similar studies of hypothalamus (Houlahan et al., 2014) and liver (Yao et al., 2012) that employed similar treatments. A Venn diagram