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Hepatic transcriptomic responses to TCDD in dioxin-sensitive and dioxin-resistant rats during the onset of toxicity

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ABSTRACT

The dioxin congener 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) causes a wide range of toxic effects in rodent species, all of which are mediated by a ligand-dependent transcription-factor, the aryl hydrocarbon receptor (AHR). The Han/Wistar (*Kuopio*) (H/W) strain shows exceptional resistance to many TCDD-induced toxicities; the LD_{50} of >9600 μg/kg for H/W rats is higher than for any other wild-type mammal known. We previously showed that this resistance primarily results from H/W rats expressing a variant AHR isoform that has a substantial portion of the AHR transactivation domain deleted. Despite this large deletion, H/W rats are not entirely refractory to the effects of TCDD; the variant AHR in these animals remains fully competent to up-regulate well-known dioxin-inducible genes. TCDD-sensitive (Long–Evans, L–E) and resistant (H/W) rats were treated with either corn-oil (with or without feed-restriction) or 100 μg/kg TCDD for either four or ten days. Hepatic transcriptional profiling was done using microarrays, and was validated by RT-PCR analysis of 41 genes. A core set of genes was altered in both strains at all time points tested, including *CYP1A1*, *CYP1A2*, *CYP1B1*, *Nqo1*, *Aldh3a1*, *Tiparp*, *Exoc3*, and *Inmt*. Outside this core, the strains differed significantly in the breadth of response: three-fold more genes were altered in L–E than H/W rats. At ten days almost all expressed genes were dysregulated in L–E rats, likely reflecting emerging toxic responses. Far fewer genes were affected by feed-restriction, suggesting that only a minority of the TCDD-induced changes are secondary to the wasting syndrome.

Introduction

Chlorinated dioxins are produced by a variety of industrial processes, including incineration, recycling of electrical and electronic devices, and the manufacture of pesticides. While our knowledge of the effects of dioxin-exposure on humans arises mainly from accidents along with incidents of occupational exposure and epidemiological studies (Schecter et al., 2006), the biochemical and toxic effects have been extensively investigated in many laboratory animal models. In particular, studies have focused on 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) because this congener has the highest toxicity for a wide variety of endpoints (Pohjanvirta and Tuomisto, 1994). TCDD causes acute lethality in laboratory animals at doses as low as ~1 μg/kg for guinea pigs and is preceded by a wasting syndrome (Birnbaum and Tuomisto, 2000). A wide range of other short-term toxic effects is known, including thymic atrophy, necrotic or hypertrophic hepatic lesions, a variety of endocrine imbalances and immuno-suppression (reviewed in Pohjanvirta and Tuomisto, 1994).

All of the dioxin-induced toxicities examined to date have been proven to be mediated by a ligand-dependent transcription-factor, the aryl hydrocarbon receptor (AHR). Ligand binding induces disaggregation of the AHR from its cytoplasmic complex (Lin et al., 2007; McMillan and Bradfield, 2007), hetero-dimerization, and translocation into the nucleus. In the nucleus, the AHR binds to cognate response elements denoted aryl hydrocarbon response elements (AHREs) (Denison et al., 1988; Denison and Whitlock, 1995; Boutros et al., 2004; Sogawa et al., 2004) and regulates mRNA transcription through multiple mechanisms (Hankinson, 2005; Beischlag et al., 2008). Mice in which the AHR has been genetically ablated are refractory to dioxin-induced toxicities (Fernandez-Salguero et al., 1995; Schmidt et al., 1996; Mimura and Fujii-Kuriyama, 2003) and to dioxin-induced transcriptional alterations (Tijet et al., 2006; Boutros et al., 2009). If AHR expression is retained but the protein is mutated to prevent nuclear translocation

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(Bunger et al., 2003) or binding to AHREs (Bunger et al., 2008), mice again become refractory to dioxin-induced toxicities. Furthermore, mice hypomorphic for the AHR's hetero-dimerization partner, ARNT, are phenotypically non-responsive to dioxins (Walisser et al., 2004). Taken together, these genetic manipulations indicate that association of ligand-activated AHR with DNA is critical for dioxin-toxicities.

The central importance of AHR–DNA interactions has led several groups to search for AHR target genes using microarray technologies. However, making the link between genes whose mRNA abundances are altered by exposure to dioxins and specific toxic end-points has been difficult. Studies of human cell-cultures (Puga et al., 2000; Frueh et al., 2001; Ishida et al., 2002; Martinez et al., 2002), mouse tissues (Boverhof et al., 2004, 2005; Karyala et al., 2004; Moennikes et al., 2004; Tijet et al., 2006; N'Jai et al., 2008) and rat tissues (Fletcher et al., 2005; Boverhof et al., 2006; Boutros et al., 2008; Moffat et al., 2010) have consistently shown that hundreds to thousands of genes respond to dioxins. Identifying the specific genes whose dysregulation is causal of toxicity from among this plethora has proven challenging (Okey, 2007).

Accordingly, several groups have exploited genetic models to winnow down lists of AHR-mediated mRNA changes to those that are most likely to be responsible for toxic outcomes. Two models have received particular focus. First, the rat and mouse animal models have broadly similar toxicologic responses to TCDD exposure. By comparing the set of genes altered by TCDD exposure in each species it ought to be possible to identify a common set of genes that mediate toxicities in both species. This approach has identified a core transcriptional response to TCDD at early time-points, along with a surprisingly marked species-dependency (Boverhof et al., 2006; Boutros et al., 2008). The second animal model involves the Han/Wistar (*Kuopio*) (H/W) strain of rat (Pohjanvirta and Tuomisto, 1987; Pohjanvirta et al., 1987). H/W rats show exceptional resistance to many TCDD-induced toxicities, especially acute lethality where the LD_{50} of >9600 μg/kg for H/W rats is higher than for any other known wild-type mammal (Pohjanvirta and Tuomisto, 1994). This resistance is strongly linked to expression of a variant AHR isoform that has a substantial portion of the transactivation domain (TAD) deleted by utilization of cryptic splice-sites (Pohjanvirta et al., 1998, 1999; Tuomisto et al., 1999). Surprisingly, despite this large deletion, H/W rats are not entirely refractory to the molecular and biochemical effects of TCDD exposure (Pohjanvirta et al., 1995; Simanainen et al., 2002).

We recently compared the genes perturbed by TCDD exposure in H/W rats to those in dioxin-sensitive Long–Evans (*Turku/AB*) rats at early time-points. Within 24 h of TCDD exposure several hundred genes are altered in each strain, with many of these displaying strain-specific expression patterns (Franc et al., 2008; Moffat et al., 2010). Here, we extend those studies and profile the transcriptional-response to TCDD of sensitive and resistant rat strains at time-points during and immediately after the onset of toxic phenotypes. Exploiting the resistant rat model at these later time-points allows us to identify genes and pathways altered in resistant or sensitive rat strains only. Genes whose mRNA levels are altered differently between dioxin-sensitive and dioxin-resistant rats are prime candidates to explain dioxin-induced toxicities.

Methods

Microarray hybridization. The design of this experiment is given in Fig. 1. We examined male rats of two strains, Long–Evans (*Turku/AB*) and Han/Wistar (*Kuopio*), abbreviated as L–E and H/W respectively. Each animal was treated with either 100 μg/kg TCDD or corn-oil vehicle (4 mL/kg by gavage) at the age of 11–15 weeks. To this end, the rats were arranged in order by weight and

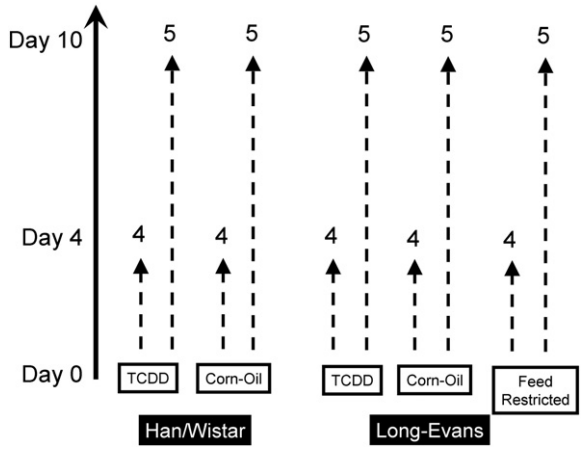

Fig. 1. Experimental design. We analyzed responses of dioxin-resistant Han/Wistar (*Kuopio*) and dioxin-sensitive Long–Evans (*Turku/AB*) rats to TCDD (henceforth H/W and L–E rats, respectively). Both H/W and L–E rats were treated with either corn oil vehicle or TCDD (100 μg/kg body weight) at time zero. Vehicle-treated L–E rats were either allowed to feed *ad libitum* or placed on a feed-restricted diet to mimic TCDD-induced reduction in food intake. Four days after dosing, four animals from each treatment group were euthanized, their livers excised, RNA extracted and hybridized to RAE230-2 arrays for transcriptomic profiling. Ten days after dosing, five animals from each treatment group were similarly euthanized and analyzed. In total 45 animals were assessed: 18 H/W and 27 L–E.

assigned to the experimental groups by a pre-designed scheme which aimed to match the group means for body weights. The rats were euthanized by decapitation either four days or ten days after treatment. The rats were housed singly in suspended stainless steel wire-mesh cages and had free access to R36 feed (Ewos, Södertälje, Sweden) and water. The feed was pelleted for the H/W rats but powdered for L–E rats (to enable accurate monitoring of feed consumption and its comparison with that in feed-restricted L–E rats). The animal room was artificially illuminated with a 12/12 h light/dark cycle (lights on at 7:00 h), and the rats were fed during the early light hours daily. The temperature was maintained at 21.5 ± 1 °C and humidity at 55 ± 10%. Because TCDD exposure induces a wasting syndrome in L–E rats, we assessed gene-wise mRNA abundances in corn-oil treated/feed-restricted L–E rats at both time-points. The feed-restricted controls were provided feed according to a pre-designed protocol based on previous data (Pohjanvirta et al., 2008) and intended to mimic the feed consumption of L–E rats treated with 100 μg/kg. In total 45 animals were assessed. All animal study plans were approved by the Animal Experiment Committee of the University of Kuopio and the Provincial Government of Eastern Finland. After euthanasia, the liver of each animal was excised, sliced, and snap-frozen. The tissues were later homogenized and total RNA was extracted using Qiagen RNeasy kits according to the manufacturer's instructions (Qiagen, Mississauga, Canada). Total RNA yield was quantified by UV spectrophotometry and RNA integrity was verified using an Agilent 2100 BioAnalyzer (Agilent Technologies, Santa Clara, CA). The isolated RNA was assayed on Affymetrix RAE230-2 arrays at The Centre for Applied Genomics at The Hospital for Sick Children (Toronto, Canada) following standard manufacturer's protocols, with each animal being assessed on a single microarray. cDNA was reverse transcribed using the SuperScript Choice System (Invitrogen Life Technologies Corp.). To prime first strand cDNA synthesis, the GeneChip T7-Oligo(dT) Promoter Primer Kit (Affymetrix Inc., St. Clara, CA) was used. Double-stranded cDNA was synthesized by reverse transcription of 10 μg total RNA using the Invitrogen SuperScript Kit as recommended by the manufacturer. The cDNA was then purified with the Affymetrix GeneChip sample cleanup module. Purified cDNA was later used as template for the synthesis of biotin-labeled target cRNA (n = 4 for each time