AN ACTIVATED ARYL HYDROCARBON RECEPTOR CAUSES DNA DAMAGE AND STRESS RESPONSE IN MOUSE LIVER

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Introduction:
Cytochrome P450s (CYP) constitute a superfamily of enzymes crucial for the metabolism of a diverse group of compounds, including endobiotics and xenobiotics[1]. Three genes, *CYP1A1, CYP1A2* and *CYP1B1,* are members of the CYP1 family [1]. All of them are all transcriptionally controlled by the aryl hydrocarbon receptor-aryl hydrocarbon receptor nuclear translocator (AhR-ARNT) pathway [2]. The cytoplasmic AhR is a member of the basic-helix-loop-helix PAS (Per/ARNT/Sim) family of nuclear transcription factors. Upon ligand activation it forms a heterodimer with the ARNT protein, and activates transcription of certain genes including *CYP1* genes by binding to xenobiotic-responsive elements (XRE) located in the 5´-flanking region of those genes [3, 4].
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) was classified as a group I carcinogen by the International Agency for Research on Cancer (IARC) [5]. It acts through an aryl hydrocarbon receptor (AhR) mediated mechanism in humans and experimental animals [6]. It has been hypothesised that TCDD may be indirectly genotoxic via generation of reactive oxygen species (ROS) by inducing these CYP enzymes. This may lead to DNA damage via direct interaction or via generation of reactive metabolites from endogenous compounds [7, 8].
We determined the amount of the oxidative damage marker 8-oxo-2´-deoxyguanosine (8-oxo-dG) using HPLC-MS/MS in mouse liver after treatment of the animals with TCDD. We also measured 8-oxo-dG in livers of a transgenic mouse model, which expresses a constitutively active AhR (CA-AhR). Transcription of the AhR gene battery in this mouse model is stimulated in the absence of any ligand [9].
Microarrays, representing genes involved in stress and toxicity, were performed with hepatic RNA isolated from TCDD-treated, and from transgenic mice and compared to wild type and vehicle controls, respectively.

Materials & Methods:
Experimental design: Wildtype C57/B6 mice were obtained from Charles River (Sulzfeld, Germany). CA-AhR mice were kindly provided by Prof. L. Poellinger, Karolinska Institute, Stockholm, Sweden [9, 10]. Mice were housed in groups of four to eight animals in macrolon cages with pelleted feed and tap water available ad libitum. Male and female mice weighing ~30 g were treated with 1µg/kg TCDD or corn oil (vehicle control) by i.p. injection on day 0 and killed by decapitation after 96 hours. Livers were removed and immediately shock frozen in liquid nitrogen.
EROD assay: Liver homogenates were prepared from frozen liver tissue with a PTFE pestle in a glass tube homogenizer followed by centrifugation at 1,000 x g. Homogenization was performed using NaPi buffer supplemented with protease inhibitor cocktail (Sigma) to prevent protein degradation. The catalytic activity of CYP1A enzymes was measured as 7-ethoxyresorufin-O-deethylase (EROD) activity according to the method of Kennedy and Jones [11].
Quantification of 8-oxo-dG: Isolation of genomic DNA from liver was performed using the high salt method. First, solutions were incubated with nuclease P1 (Sigma) and alkaline phosphatase (Roche). Proteins and incompletely hydrolyzed DNA were separated by centrifugation through Amicon Ultrafree®-filters. The resulting hydrolyzed DNA solution was injected into the HPLC-MS/MS system.
HPLC-MS/MS: The HPLC-MS/MS system consisted of an Agilent 1100 LC binary pump and autosampler (Agilent 110 Autosampler) with an API 3000 mass spectrometer (Applied Biosystems). HPLC separations were achieved using a AQ 12S051502 QT, 150 x 2.1 mm column (YMC). The mobile phase consisted of 10 mM ammonium acetate, pH 4.3, and methanol. Electrospray ionization was carried out in positive ion mode using nitrogen as the nebulising gas. Linear HPLC-MS/MS calibration curves with external 8-oxo-dG standard were obtained over the range of 0.1–10 pg/µl. For HPLC-MS/MS analysis in multiple reaction monitoring (MRM) mode transitions m/z = 284.1/186.3 (8-oxo-dG) were recorded. Transitions for unmodified 2´-deoxynucleosides were also monitored; dG (m/z = 268.1/152.1)
Microarrays:
Total RNA was isolated from liver slices using an RNeasy mini kit (Qiagen; Hilden, Germany). The RNA was enzymatically converted into a biotinylated cDNA target with the AmpoLabeling-LPR™ Kit (Superarray Frederick, USA) in a standard thermal cycler. The labeled targets were hybridized to cDNA GEArray microarrays in a standard hybridization oven. Detection of the chemiluminescent microarrays was performed using a CCD camera. The data were acquired and analyzed using GEarray´s software package.

Results:

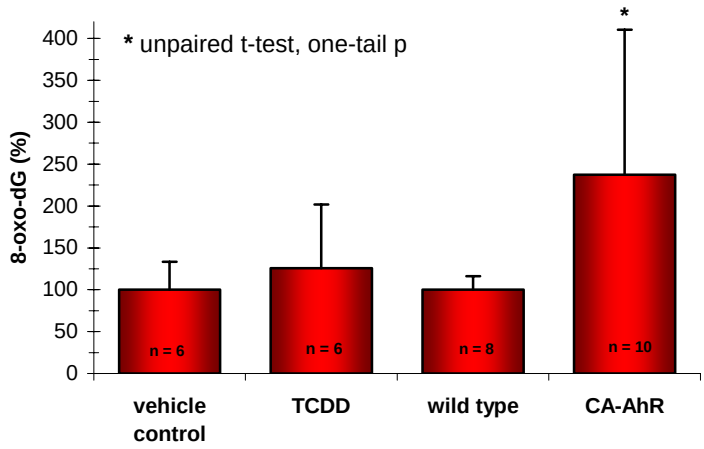

Figure 1: 8-oxo-dG levels in genomic liver DNA samples

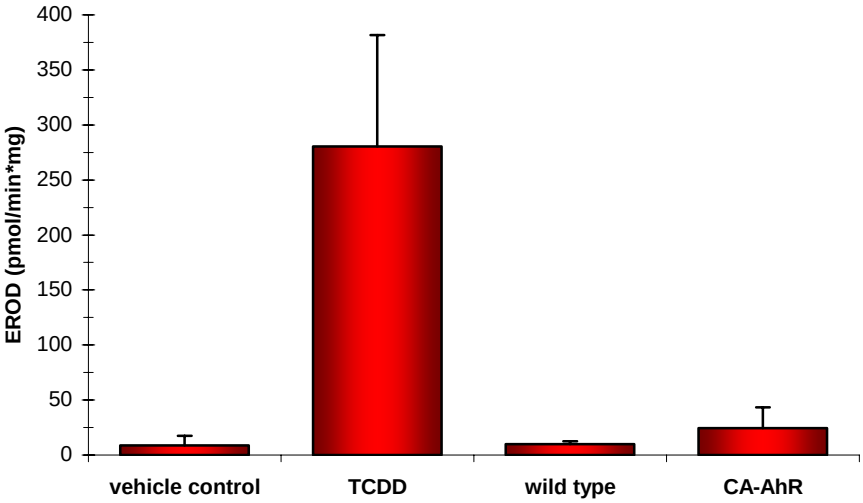

Figure 2: EROD activity in liver homogenates

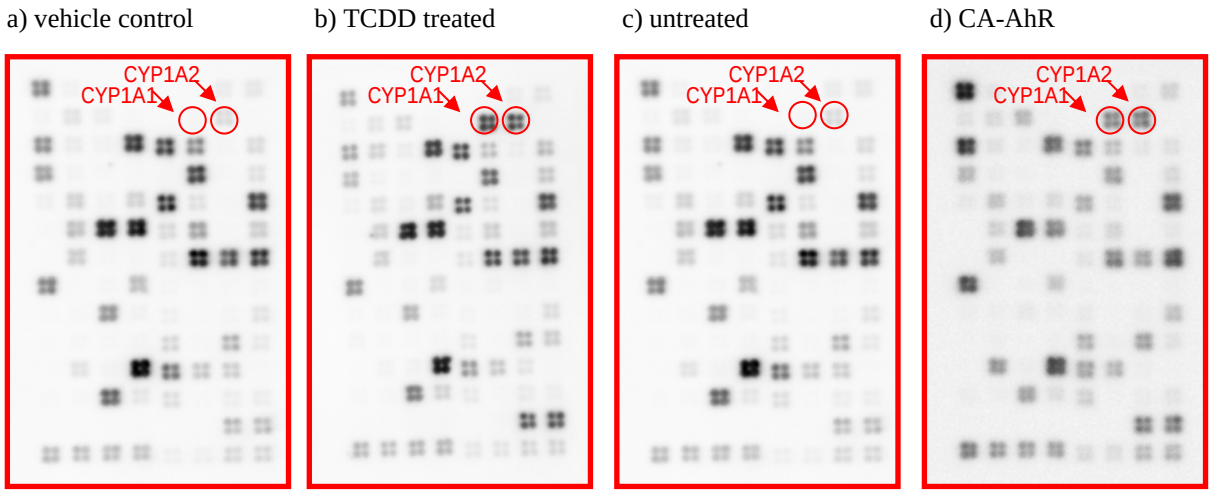

Figure 3: Representative cDNA array membranes of mouse liver mRNA-derived cDNAs

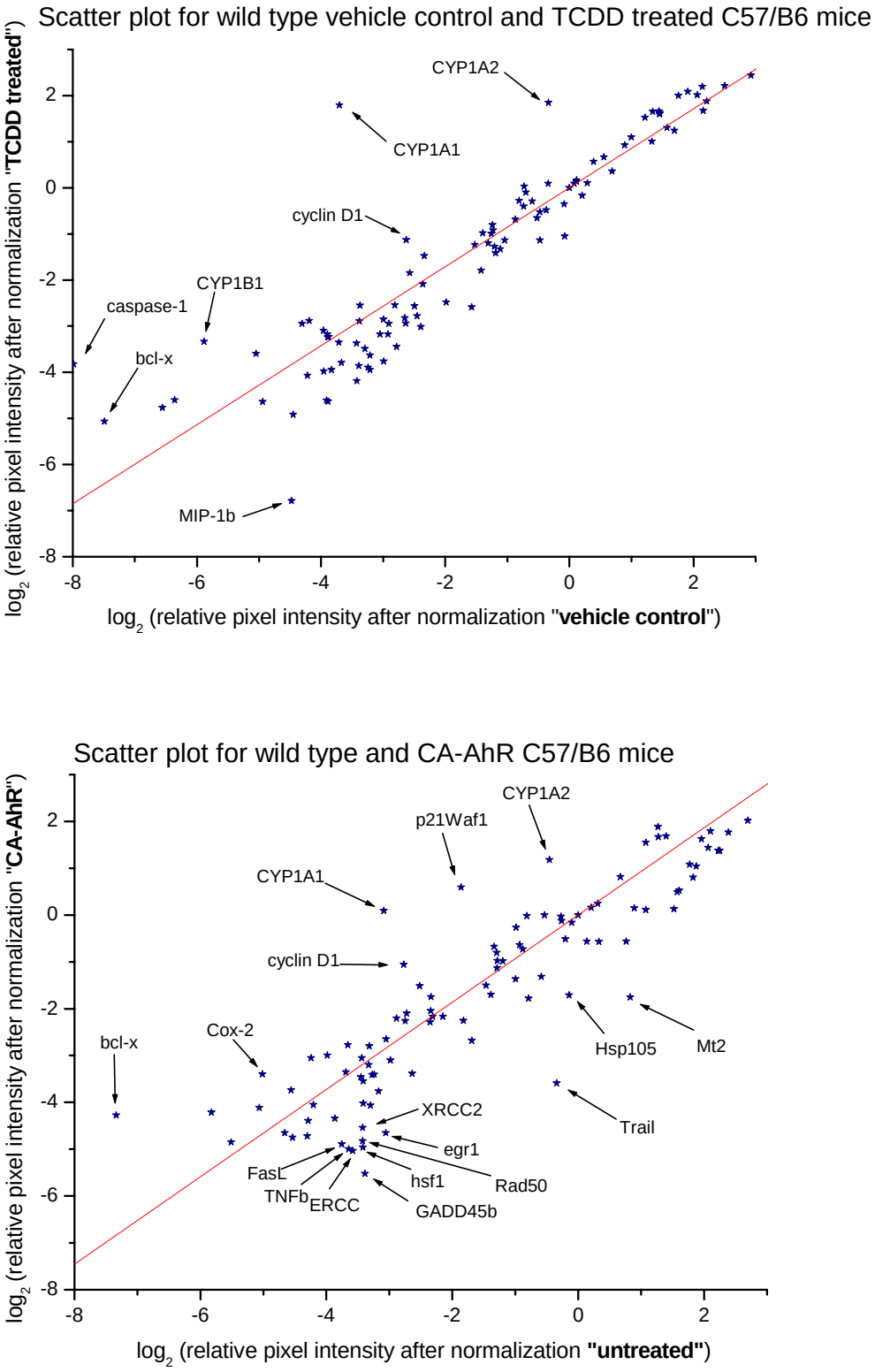

Figure 4: Scatter plot results of cDNA arrays analysing gene expression

Gene	*CA-AhR*	*TCDD*	*function*
CYP1A1	⇧ ⇧	⇧ ⇧	drug metabolism
CYP1A2	⇧ ⇧	⇧ ⇧	drug metabolism
Cyclin D1	⇧	⇧	cell cycle
CYP1B1		⇧ ⇧	drug metabolism
p21 ^{Waf1}	⇧ ⇧		apoptosis regulation
bcl-x	⇧ ⇧	⇧	apoptosis regulation
caspase-1		⇧ ⇧	apoptosis regulation
Cox-2	⇧ ⇧		inflammation
MIP-1b		⇩ ⇩	inflammation
Fas-L	⇩		apoptosis regulation
GADD45	⇩ ⇩		growth arrest and DNA damage response
Trail	⇩ ⇩		apoptosis regulation
Mt2	⇩ ⇩		oxidative & metabolic stress
TNFbeta	⇩		inflammation
ERCC	⇩		DNA damage & repair
Hsp105	⇩		heating stress response

Table 1: selected up ⇧ /down ⇩ regulated genes after analysis of cDNA microarrays

Discussion:

96 h after treatment of wildtype mice with 1 µg/kg TCDD, slightly increased levels of 8-oxo-dG were observed in hepatic DNA. In the livers of CA-AhR mice basal 8-oxo-dG levels were significantly higher than in untreated or TCDD-treated wild type mice. In contrast, the catalytic activity of hepatic CYP1A enzymes, measured as EROD activity, was much lower in CA-AhR than in TCDD-treated wild type mice. Interestingly, microarray data indicated that much more genes were differentially expressed in the CA-AhR compared to TCDD-treated mice. Besides an explicit induction of CYP1A1, differential expression of hepatic genes involved in apoptotic processes, e.g. Bcl-2 or Bax was observed for both the TCDD-treated and the transgenic mice.

It must be noticed that wildtype mice were treated with an acute dose of TCDD for only 96 hours. Therefore, it is difficult to compare these results with those from the transgenic mouse model which expresses the AhR constitutively over lifetime. Nevertheless some differentially expressed genes, especially genes involved in apoptotic processes, were detected both in the TCDD-treated, and in transgenic mice and are possibly regulated as a consequence of an activated AhR.

In conclusion, a sustained or permanent activation of the AhR leads on the one hand to increased oxidative hepatic DNA damage. On the other hand apoptotic pathways seem to be modified, possibly in response to oxidative stress.

References:

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