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2,3,7,8-Tetrachlorodibenzo-p-dioxin enhances CCl₄-induced hepatotoxicity in an aryl hydrocarbon receptor-dependent manner

Alejandro Mejia-Garcia¹, Esmeralda M. Sanchez-Ocampo¹, Silvia Galindo-Gomez², Mineko Shibayama², Octavio Reyes-Hernandez³, Simon Guzman-Leon⁴, Frank J. Gonzalez⁵, and Guillermo Elizondo¹

¹Departamento de Biología Celular, CINVESTAV-IPN, Zacatenco, México D. F., C.P 07360

²Departamento de Infectómica y Patogénesis Molecular, CINVESTAV-IPN, Zacatenco, Mexico D. F., C.P 07360, ³Laboratorio de Genética y Diagnóstico Molecular, Unidad de Investigación del Hospital Juárez de México, ⁴Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, National Institutes of Health, Bethesda, Maryland ⁵Laboratory of Metabolism, NCI, National Institutes of Health, Bethesda, Maryland

Abstract

Cytochrome P4502E1 (CYP2E1) is involved in the biotransformation of several low molecular weight chemicals and plays an important role in the metabolic activation of carcinogens and hepatotoxins such as CCl₄. Induction of CYP2E1 is exerted mainly at posttranscriptional levels through mRNA and protein stabilization, and there is little evidence of xenobiotic induction at the transcriptional level. Previously, we reported microarray analysis data suggesting a decrease in *Cyp2e1* gene expression on *Ahr*-null livers when compared to wild-type mouse livers.

The goal of the present study was to determine whether 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) increased mouse CYP2E1 levels in an AhR-dependent manner and the impact on CCl₄-induced hepatotoxicity.

TCDD treatment induced CYP2E1 mRNA and protein levels in mouse liver, and this effect was aryl hydrocarbon receptor (AhR)-dependent.

Moreover, TCDD pre-treatment increased the CCl₄-induced alanine aminotransferase (ALT) activity, the extent of CCl₄-induced necrosis, and the number of sinusoidal cells in wild-type animals, while this potentiating effect was not observed in *Ahr*-null mice.

In conclusion, this study revealed that TCDD, probably in an AhR-dependent manner, exacerbated CCl₄-induced hepatotoxicity through induction of CYP2E1.

Keywords

TCDD; AhR; CYP2E1; CCl₄

Introduction

Cytochrome P4502E1 (CYP2E1) is involved in the biotransformation of several low molecular weight chemicals, many of which are organic solvents. This enzyme has been extensively studied because it plays an important role in the metabolic activation of precarcinogens, such as benzene and nitrosamines, as well as hepatotoxins, including carbon tetrachloride (CCl₄) and acetaminophen (Wong et al., 1998; Cheung et al., 2005). Several endogenous CYP2E1 substrates also have been identified, including lipid peroxidation products, ketones, and fatty acids; and it was suggested that they play a role in gluconeogenesis (Lieber, 2004).

CYP2E1 is constitutively expressed in the liver and several other tissues. CYP2E1 induction by various chemicals is exerted mainly at post-transcriptional level, and there is little evidence of xenobiotic induction at the transcriptional level. Mouse, rat, and human CYP2E1 transcription is mainly under the control of hepatocyte nuclear factor 1 α (HNF1 α) (Cheung et al., 2003). Other studies have shown that mouse CYP2E1 expression is also regulated by β -catenin (Sekine et al., 2006) and leptine (Leclercq et al., 2000). On the other hand, aryl hydrocarbon receptor (AhR) ligands such as 3-methylcholanthrene, β -naphthoflavone and pyridine increase CYP2E1 transcription and activity (Anderson and Angel, 1980; Mostafa et al., 1981; Kim et al., 1988; Sinal et al., 1999; Iba et al., 2000), suggesting a role for this transcription factor in the regulation of CYP2E1 expression. More recently, it was reported that TCDD induce CYP2E1 expression in H9c2 cells (Zordoky and El-Kadi, 2010).

AhR, a member of the basic helix-loop-helix-Per-Arnt-Sim (bHLH-PAS) transcription factor family, is a ligand-activated receptor that mediates the toxicity of environmental pollutants, such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (Okey et al., 1994; Whitlock, 1999). Upon binding to TCDD, AhR translocate to the nucleus, dimerises with AhR nuclear-translocator protein (ARNT), binds dioxin-responsive elements (DREs, also known as xenobiotic-responsive elements or XREs), and upregulates the expression of a battery of genes encoding xenobiotic-metabolizing enzymes, such as cytochrome P450s (e.g. CYP1A1, CYP1A2, CYP1B1), NAD(P)H quinone oxidoreductase, and a form of UDP-glucuronosyl-transferase-6 (Gonzalez and Fernandez-Salguero, 1998). Although AhR may function as part of an adaptive chemical response, several studies have suggested that this transcription factor could have important functions in liver and cardiac development, cell proliferation, circadian rhythmicity, cholesterol and glucose metabolism (Fernandez-Salguero et al., 1996; Gonzalez and Fernandez-Salguero, 1998; Elizondo et al., 2000; Sato et al., 2008), as well as in the ubiquitin-proteasome system (Reyes-Hernandez et al., 2010). Previously, we reported microarray analysis data suggesting that several genes encoding for metabolizing enzymes are under AhR regulation (Reyes-Hernandez et al., 2010). Several years ago, some of these were identified as part of the AhR battery, including *cyp1a1* and *cyp1a2*. The microarray data also indicated that *akr1c13*, *akr1e1*, *cyp39a1*, *cyp4a12a* and *cyp2e1* gene expression was down regulated in *Ahr*-null mouse livers when compared to wild-type livers.

Given the role of CYP2E1 in the metabolism of several toxins and carcinogens, the effect of xenobiotics on CYP2E1 liver expression is of significant interest. Therefore, the goal of the present study was to determine whether TCDD increased mouse CYP2E1 levels in an AhR-dependent manner and the impact on CCl₄-induced hepatotoxicity.

Materials and methods

Materials

TCDD (99.1% purity) was purchased from AccuStandard (New Haven, CT), carbon tetrachloride (CCl₄, 99.9% purity) was purchased from J.T. Baker (Austin, TX), Tris-HCl and sodium dodecylsulfate were purchased from Invitrogen (Camarillo, CA), Tween 20 and Nonidet P-40 were purchased from Sigma-Aldrich (St. Louis, MO), and glycine was purchased from Usbio (Marblehead, MA).

Animals

The *Ahr*-null mice (C57BL/6N) described previously (Fernandez-Salguero et al., 1995) were genotyped by PCR as reported elsewhere (Rodriguez-Sosa et al., 2005). Wild-type (WT) littermates were used as control mice. Animals were randomly distributed ($n = 5$), housed in a pathogen-free facility, fed with autoclaved Purina rodent chow (St. Louis, MO), and provided with water *ad libitum*. All animal studies were performed according to the Guide for the Care and Use of Laboratory Animals, as adopted and enforced by the US National Institutes of Health and the Mexican Regulation of Animal Care and Maintenance (NOM-062-ZOO-1999, 2001).

Treatments

Three treatment groups were formed (male mice, aged 6–8 weeks, $n = 5$). Group 1 mice were given a single intraperitoneal (i.p.) dose of TCDD at 80 µg/kg body weight and were sacrificed 72 h later. Group 2 mice were given a single i.p. dose of CCl₄ at 1.59 g/kg body weight and were sacrificed 24 h later. Group 3 mice were given a single i.p. dose of TCDD at 80 µg/kg body weight and 48 h later were given a single i.p. dose of CCl₄ at 1.59 g/kg; the mice were sacrificed 24 h later. Controls for each group were injected with corn oil vehicle only. After treatments, mice were euthanized by cervical dislocation, and livers were removed, frozen in liquid nitrogen, and stored at -70°C . For histopathology analysis, representative liver fragments were fixed with 10% formaldehyde in phosphate-buffered saline, pH 7.2–7.4.

Real-time quantitative PCR (rtPCR) analysis

Briefly, total RNA was prepared from mouse liver using the TRIzol reagent according to the manufacturer's instructions (Invitrogen; Camarillo, CA). RNA was quantified spectrophotometrically at OD₂₆₀. Subsequently, RNA integrity was evaluated by electrophoresis of RNA samples on 1% agarose gels. cDNA for the rtPCR assay was prepared from 2 µg of total RNA using the SuperScript First-Strand Synthesis reagent (Invitrogen; Camarillo, CA) and oligo dT (Invitrogen; Camarillo, CA). PCR experiments were conducted using a Step One Real Time PCR System (Applied Biosystems; Branchburg, NJ) and analysed using the comparative threshold cycle (Ct) method, as

described previously (Medina-Diaz and Elizondo, 2005). The mRNAs encoding CYP2E1 or 18S ribosomal RNA (rRNA, endogenous) were amplified in a single PCR experiment to allow for normalization of the mRNA data. The PCR mixture contained 2 µl of cDNA, 0.9 µM primers, and 0.25 µM probes in 1X TaqMan Universal PCR Master Mix (Applied Biosystems; Foster City, CA). The primer and probe sequences used for CYP2E1 were as follows: 5'-CCAAAGAGAGGCACACTTCCT-3' (forward), 5'-GCCAATCAGAAAGGTAGGGTCAAAA-3' (reverse), and CTGGCCTTTGGTCTTTT (FAM probe). The primers and probe for CYP1A1 were: 5'-GCCTTCATTCTGGAGACCTTCC-3' (forward), 5'-CAATGGTCTCTCCGATGC-3' (reverse), and CAGGACATTTGAGAAGGGCCACATC (FAM probe).

In silico analysis

The mouse *cyp2e1* gene promoter was analysed using the following web-based bioinformatic tools: Genomatix (http://www.genomatix.de/cgi-bin/matinspector_prof/mat_fam.pl?s=eabd9a3f188f5b276aaa00a3445cdcbe, 2009) and CBRC (<http://www.cbrc.jp/research/db/TFSEARCH.html>, 2009).

Western blot analysis

Liver fragments were homogenised in buffer containing 10 mM Tris-HCl (pH 7.4), 1% Nonidet P-40, 10 µg/ml aprotinin, 10 µg/ml leupeptin, 10 µg/ml soybean trypsin inhibitor, and 1 mM PMSF. Protein concentrations were determined using the Bradford reaction (BioRad; Hercules, CA). Aliquots (20 µg) were solubilised in sample buffer [60 mM Tris-HCl, pH 6.8; 2% sodium dodecylsulfate (SDS); 20% glycerol; 2% mercaptoethanol; 0.001% bromophenol blue] and subjected to 10% SDS-polyacrylamide gel electrophoresis. Protein extracts were transferred to a nitrocellulose membrane using a mini trans-blot (Bio-Rad; Hercules, CA). The transfer was performed at a constant voltage of 80 V for 2 h in transfer buffer (48 mM Tris-HCl, 39 mM glycine, pH 8.3; 20% methanol). Following the transfer, membranes were blocked overnight at 4°C in the presence of 2% nonfat dry milk and 0.5% bovine serum albumin (BSA) in blocking buffer (25 mM Tris-HCl, pH 7.5; 150 mM NaCl) and subsequently incubated at 4°C for 3 h with goat polyclonal anti-CYP2E1 (1:10,000; Abcam; Cambridge, MA), or actin (1:5000; Zymed; San Francisco, CA) diluted in buffer (25 mM Tris-HCl, pH 7.5; 150 mM NaCl; 0.1% Tween 20; 0.05% nonfat dry milk; 0.05% BSA). After washing, the membranes were incubated with the corresponding horseradish peroxidase (HRP)-conjugated secondary antibody, HRP-rabbit anti-goat IgG (1:10,000; Pierce; Rockford, IL), for 2 h at 4°C. The membrane was washed, and the immunoreactive protein was detected using an ECL western blotting detection kit (Amersham; Arlington Heights, IL). The integrated optical density of the bands was quantified using scanning densitometry (GS-800 Calibrated Densitometer; BioRad; Hercules, CA).

Determination of serum aminotransferase activities

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined enzymatically by the rate of NADPH oxidation using Randox reagents according to the manufacturer's instructions (Randox; Antrim, UK).

Liver histology

For each treatment group, a representative section was excised from the liver. Tissues were fixed in 10% formaldehyde in phosphate-buffered saline for 48 h, washed in water, dehydrated in alcohol, and embedded in paraffin. Liver sections (5 μ m in thickness) were stained with hematoxylin-eosin (H&E) for histological examination under a light microscope (Nikon H550L).

Statistical analysis

Results are presented as the mean values $\pm$ the standard deviation (SD) or the standard error (SE). The statistical significance of the data was evaluated using the Mann–Whitney’s U–Wilcoxon rank or the Student’s *t*-test, as appropriate. In all cases, the differences between animal groups were considered to be statistically significant when the *p* value was less than 0.05.

Results

Recently, DNA microarray analysis revealed a decrease in CYP2E1 expression on *Ahr*-null livers when compared to wild-type mouse livers (Reyes-Hernandez et al., 2010) (Table 1). In the present study, we determined whether Ahr plays a role in the modulation of CYP2E1 expression levels. In order to measure the efficacy of TCDD treatment, CYP1A1 mRNA levels were determined. As expected, an induction of CYP1A1 expression was observed only in the wild-type mouse (Figure 1A). No differences were observed on basal CYP2E1 mRNA levels, between wild-type and *Ahr*-null mice. However, TCDD treatment resulted in a five-fold induction of CYP2E1 mRNA levels only in the wild-type mice (Figure 1B). These data suggest that the induction of *cyp2e1* gene expression after TCDD treatment is Ahr-dependent. In order to identify putative XREs in the mouse *cyp2e1* gene promoter, *in silico* analysis was performed. Common regulatory sequences, such as the TATA box, HNF-3b, GATA-2, Oct-1, AP1, and C/EBP, were located between position-2046 and the putative translation start site. However, no XREs were identified (data not shown).

Next, we examined whether the induction of CYP2E1 mRNA causes increased protein levels. After TCDD treatment, a four-fold induction of CYP2E1 protein levels was observed in wild-type mice. In contrast, this effect was not detected in the livers of *Ahr*-null mice. Moreover, TCDD treatment resulted in a light but significant decrease of the immunoreactive CYP2E1 protein level in *Ahr*-null livers (Figure 2). In addition, TCDD treatment is known to result in typical wasting syndrome and loss of body weight, which may cause increased CYP2E1 expression due to the accumulation of ketone bodies. Therefore, the body weight of the animals was monitored throughout TCDD treatment. No change was observed during the study, suggesting that the observed induction of CYP2E1 mRNA might be a direct result of AhR activation (Figure 3).

It is well established that CYP2E1 mediates the toxicity of several agents, such as acetaminophen and CCl₄. Therefore, we explored whether TCDD pretreatment magnifies the CCl₄ toxicity. Initially, TCDD hepatotoxicity was determined. In both wild-type and *Ahr*-null mice, increased serum aminotransaminase activities were observed after TCDD

treatment. For both ALT and AST, the increased activity was higher in wild-type mice compared to *Ahr*-null mice (Figure 4). Histopathological analysis showed that after TCDD treatment, the wild-type liver presents pyknotic cells and mild necrosis. In contrast, *Ahr*-null mice did not exhibit apparent histopathological changes (Figure 5). Next, the effect of TCDD pretreatment on CCl₄ hepatotoxicity was determined (Figure 6). Treatment with CCl₄ alone resulted in marked increases of serum ALT and AST activities in both wild-type and *Ahr*-null mice. There were approximately 800- and 113-fold increases in ALT and AST activities, respectively, in wild-type mice when compared to their corn oil-treated counterparts (Figure 4). In contrast, after CCl₄ treatment, *Ahr*-null mice presented lower increases in ALT and AST activities (580-fold and 101-fold, respectively). In addition, TCDD pretreatment increased the CCl₄-induced ALT activity by 1643-fold in wild-type animals compared to the corn oil control group, while this potentiating effect was not observed in *Ahr*-null mice. Although TCDD pretreatment increased CCl₄-induced AST activity in wild-type mice, this effect was slightly significant. No differences were observed in *Ahr*-null animals (Figure 6).

It is known that CCl₄ induces hepatotoxicity and is characterized by centrilobular necrosis. Histopathological evaluation of liver sections from wild-type mice treated with CCl₄ showed severe centrilobular necrosis when compared to corn oil-treated wild-type animals (Figures 5 and 7). Pretreatment with TCDD increased the extent of CCl₄-induced necrosis and the number of sinusoidal cells. Although necrosis was observed in liver sections from *Ahr*-null mice treated with CCl₄, the extent of the damage was lower when compared to wild-type mice. Pretreatment with TCDD did not potentiate the CCl₄-induced necrosis or produce altered nuclei and sinusoidal damage in *Ahr*-null liver samples (Figure 7).

Discussion

Transcriptional induction of CYP2E1 is complex and not well understood. The present study shows that AhR activation by TCDD enhanced CCl₄-induced hepatotoxicity, at least in part, by increasing CYP2E1 mRNA and protein levels. Previous reports have suggested that AhR mediates CYP2E1 expression. In addition, β -naphthoflavone treatment has been shown to increase the CYP2E1 transcript levels in rat liver, lung, heart, and kidney (Sinal et al., 1999). On the other hand, pyridine, a major constituent of tobacco smoke induces CYP2E1 levels (Kim et al., 1988). This agent also induces *cyp1a1* gene expression in an AhR-dependent manner (Iba et al., 2000).

Similar to the present report, hematotoxicity induced by benzene, a CYP2E1 substrate, is mediated by AhR. Moreover, benzene treatment increases CYP2E1 expression in wild-type but not in *Ahr*-null mouse livers (Yoon et al., 2002). These reports together with the present data suggest that AhR participates in CYP2E1 induction.

However, *in silico* analysis has failed to identify XREs at the *cyp2e1* gene promoter. While the presence of a nonconsensus XREs cannot rule out, it is likely that AhR acts through a novel, XRE-independent mechanism. Indeed, several studies have demonstrated that the *cyp2e1* gene transcription control is under control by transcription factors such as HNF1 α (Cheung et al., 2003). On the other hand, a 24-h rhythm of CYP2E1 protein and mRNA

levels in mouse liver has also been reported (Matsunaga et al., 2008). The same authors have already elucidated the nature of this daily variation. Transcription of *cyp2e1* gene is rhythmically controlled by HNF1 α . This activation is negatively regulated by CRY1, a component of the circadian timing system, through its interaction with HNF1 α transcriptional complexes. More recently, others demonstrated that AhR activation disrupts CLOCK-BMAL1 transcriptional activity (Xu et al., 2010), which positively regulates *cry1* gene expression (Gekakis et al., 1998). The latter data allows us to hypothesize that the TCDD effect on CYP2E1 expression, observed in the present study, is the result of CLOCK-BMAL1 complex disruption leading to decreased *cry1* expression. Therefore, induction of CYP2E1 mRNA and protein levels occurs. It also cannot be ruled out that other transcription factors control *cyp2e1* gene expression, such as β -catenin (Sekine et al., 2006). Recently, an interaction between the AhR and the β -catenin pathway has been shown (Braeuning et al., 2011; Prochazkova et al., 2011).

Alternatively, TCDD may modify CYP2E1 mRNA levels at the post-transcriptional level. It was reported that TCDD increases stabilization of several mRNAs. In particular, TCDD induces transforming growth factor α and urokinase plasminogen activator mRNA stabilization in the human keratinocyte cell line SCC-12F (Gaido et al., 1992; Gaido and Maness, 1995). Moreover, Henley and collaborators provided evidence that AhR is involved in TCDD-stimulated IL-1 β expression through a posttranscriptional mechanism, specifically by increasing the steady state of the mRNA (Henley et al., 2004). According to a genome-wide analysis of nuclear and cytoplasmic RNA levels, the impact of TCDD-activated AhR at the posttranscriptional level includes tens, maybe hundreds, of genes (Schwanekamp et al., 2006).

Regardless of the level of control, AhR activation by TCDD resulted in increased CYP2E1 protein levels and, therefore, a potential increase in CYP2E1 activity. To assess this last possibility, the role of TCDD-activated AhR on CCl₄ toxicity was investigated. Although the magnitude of the change was low compared to other hepatotoxins, TCDD treatment increased AST and ALT activities in wild-type serum mice (2.6- and 2.2-fold induction, respectively). Surprisingly, similar results were observed, but to a lesser extent in *Ahr*-null samples. This data suggest that there are AhR-independent pathways for the toxic actions of TCDD. Consistent with this idea, Yoshioka and collaborators recently reported antiproliferative effects of TCDD in an AhR-independent way (Yoshioka et al., 2012). In agreement with previous studies (Fernandez-Salguero et al., 1996; Smith et al., 2001), liver histology showed that TCDD treatment resulted in necrosis and pycnotic nuclei, suggesting the presence of apoptotic cells in wild-type mice. In contrast, livers from *Ahr*-null mice were unaffected. As expected, CCl₄ treatment resulted in increased AST and ALT activities in both wild-type and *Ahr*-null serum. However, the serum ALT activity was potentiated by TCDD pretreatment by two-fold only in wild-type livers. In agreement with this observation, histopathology analysis showed that CCl₄ toxicity was exacerbated by TCDD treatment. These results suggest that AhR activation intensified the CCl₄ hepatotoxicity by increasing the CYP2E1 levels. Thus, exposure to AhR ligands may alter the capacity of organisms to metabolize CCl₄ into reactive metabolites that account for severe toxicity. The latter applies for all toxic compounds that are metabolized by CYP2E1, such as benzene. On the other hand, it has been shown that co-treatment with TCDD and CCl₄ results in the loss of

CYP1A2 accompanied with a possible generation of oxidative species (Kuroki et al., 1986; De Matteis et al., 1991). This may contribute to the hepatotoxicity observed in the present study.

CYP2E1 metabolizes benzene into metabolites that induce hematopoietic toxicity, aplastic anemia, and leukemia (Cronkite et al., 1984; Farris et al., 1997). Similar to the present study, Yoon and collaborators (Yoon et al., 2002) reported that *Ahr*-null mice are less susceptible to benzene toxicity than wild-type animals. Moreover, benzene treatment results in induction of CYP2E1 expression in wild-type mice, while no significant changes were observed in *Ahr*-null mouse livers.

Evidence from other laboratories and the present results suggest that AhR activation induces CYP2E1 levels. However, it is not clear whether the underlying molecular mechanism is at the transcriptional or posttranscriptional level or both.

In conclusion, the present study showed that TCDD, probably in an AhR-dependent manner, exacerbated CCl₄-induced hepatotoxicity by inducing CYP2E1 levels. Future studies are necessary to elucidate the molecular mechanism whereby AhR mediates CYP2E1 induction.

Acknowledgments

Declaration of interest

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Figure 1. TCDD increases CYP2E1 mRNA levels. Wild-type or *Ahr*-null mice were treated with TCDD (80 μ g/kg) or vehicle. After 72 h, liver samples were removed and the relative expression of CYP1A1 (A) or CYP2E1 (B) mRNA levels were determined. mRNA levels were normalised with 18S ribosomal RNA. Data represent mean values $\pm$ SD. * $p < 0.05$.

Figure 2. Effect of TCDD on CYP2E1 protein levels. Wild-type or *Ahr*-null mice were treated with TCDD (80 μ g/kg) or vehicle. After 72 h, liver samples were removed and CYP2E1 protein levels were determined. (A) Relative values for CYP2E1 protein signals were normalised with actin. (B) Representative immuno-blot image. Data represent mean values $\pm$ SE. * p < 0.05.

Figure 3. Body weights during TCDD treatment. Both wild-type and *Ahr*-null mice were treated with TCDD (80 $\mu\text{g/kg}$) as described in the Materials and Methods section, and the body weight was evaluated every day for 4 days. Data represent mean values $\pm$ SE from five animals of each genotype.

Figure 4. Serum aminotransaminase activity after TCDD treatment. Wild-type or *Ahr*-null mice were treated with TCDD (80 μ g/kg) or vehicle. After 72 h, serum levels of AST (A) or ALT (B) were determined. Data represent mean values $\pm$ SE. * p < 0.05.

Figure 5. Histopathological analysis of livers of mice treated with TCDD. Representative H&E-stained liver sections are shown from wild-type and *Ahr*-null mice treated with corn oil or TCDD (80 µg/kg) for 72 h.

Figure 6.

Aminotransaminase activity after CCl₄ and TCDD treatments. Wild-type or *Ahr*-null mice were treated with CCl₄ (1.59 g/kg) for 24 h, or co-treated with TCDD (80 µg/kg) and CCl₄ (1.59 g/kg) as indicated in Materials and methods section. After treatments, serum levels of AST (A) or ALT (B) were determined. Data represent mean values ± SE. * $p < 0.05$. Wildtype-CCl₄ vs. *Ahr*-null-CCl₄, § $p < 0.05$.

Figure 7.

Histopathological analysis of livers of mice after TCDD and CCl₄ treatments.

Representative H&E-stained liver sections are shown from wild-type and *Ahr*-null mice treated with CCl₄ (1.59 g/kg) for 24 h, or co-treated with TCDD (80 μg/kg) and CCl₄ (1.59 g/kg) as indicated in Materials and methods section.

Table 1.
Basal gene expression in Ahr-null mice livers as compared to wild-type livers*.

Metabolism of xenobiotics – *Mus musculus* (mouse).

Accession No.	Symbol	Gene name	Z score**
Phase I			
NM_010750	cyp1a1	cytochrome P450, family 1, subfamily a, polypeptide 1	-2.248441
NM_010759	cyp1a2	cytochrome P450, family 1, subfamily a, polypeptide 2	-1.873759
NM_023371	cyp2e1	cytochrome P450, family 2, subfamily e, polypeptide 1	-1.854343
NM_018887	cyp3a1	cytochrome P450, family 39, subfamily a, polypeptide 1	-2.076368
Z12223	cyp4a12a	cytochrome P450, family 4, subfamily a, polypeptide 12a	-1.705495
NM_013777	akr1c13	aldo-keto reductase family 1, member c13	-2.625138
NM_018859	akr1e1	aldo-keto reductase family 1, member e1	-2.025381
Phase II			
NM_008487	gstm2	glutathione S-transferase, mu 2	-2.585971
NM_008510	gstt1	glutathione S-transferase, theta 1	-3.387056
NM_013873	sult4a1	sulfotransferase family 4a, member 1	-2.071361

* Reyes-Hernandez et al., 2010.

** Cheadle et al., 2003.