Toxicology and Applied Pharmacology 272 (2013) 503–518

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

Knockout of the aryl hydrocarbon receptor results in distinct hepatic and renal phenotypes in rats and mice

Joshua A. Harrill [a], Renee R. Hukkanen [b], Marie Lawson [b], Greg Martin [b], Brian Gilger [c], Valerie Soldatow [d], Edward L. LeCluyse [a], Robert A. Budinsky [b], J. Craig Rowlands [b], Russell S. Thomas [a,*]

[a] The Hamner Institute for Health Sciences, Institute for Chemical Safety Sciences, RTP, NC 27709, USA
[b] The Dow Chemical Company, Midland, MI 48640, USA
[c] North Carolina State University, College of Veterinary Medicine, Raleigh, NC 27606, USA
[d] University of North Carolina, Department of Environmental Sciences and Engineering, Chapel Hill, NC 27599, USA

ARTICLE INFO

Article history:
Received 1 April 2013
Revised 29 May 2013
Accepted 24 June 2013
Available online 13 July 2013

Keywords:
Aryl hydrocarbon receptor knockout rat
Aryl hydrocarbon receptor knockout mouse
Comparison
Tissue phenotypes
Liver
Kidney

ABSTRACT

The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor which plays a role in the development of multiple tissues and is activated by a large number of ligands, including 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). In order to examine the roles of the AHR in both normal biological development and response to environmental chemicals, an AHR knockout (AHR-KO) rat model was created and compared with an existing AHR-KO mouse. AHR-KO rats harboring either 2-bp or 29-bp deletion mutation in exon 2 of the AHR were created on the Sprague–Dawley genetic background using zinc-finger nuclease (ZFN) technology. Rats harboring either mutation type lacked expression of AHR protein in the liver. AHR-KO rats were also insensitive to thymic involution, increased hepatic weight and the induction of AHR-responsive genes (*Cyp1a1*, *Cyp1a2*, *Cyp1b1*, *Ahrr*) following acute exposure to 25 μg/kg TCDD. AHR-KO rats had lower basal expression of transcripts for these genes and also accumulated ~30–45-fold less TCDD in the liver at 7 days post-exposure. In untreated animals, AHR-KO mice, but not AHR-KO rats, had alterations in serum analytes indicative of compromised hepatic function, patent ductus venosus of the liver and persistent hyaloid arteries in the eye. AHR-KO rats, but not AHR-KO mice, displayed pathological alterations to the urinary tract: bilateral renal dilation (hydronephrosis), secondary medullary tubular and uroepithelial degenerative changes and bilateral ureter dilation (hydroureter). The present data indicate that the AHR may play significantly different roles in tissue development and homeostasis and toxicity across rodent species.

© 2013 Elsevier Inc. All rights reserved.

Introduction

The aryl hydrocarbon receptor (AHR) is a ligand-activated basic helix–loop–helix/Per–ARNT–Sim (bHLH/PAS) transcription factor expressed in a variety of mammalian tissues including the liver, lung, kidney, thymus, spleen, heart, brain, ovary, testis and subpopulations of cells within the immune system (Carver et al., 1994; Chaffin et al., 2000; Li et al., 2011; Schultz et al., 2003; Veldhoen et al., 2008). In the absence of ligand, AHR is localized outside the nucleus, bound to a multiprotein complex of cytosolic binding partners consisting of HSP90, AIP, and p23 (Beischlag et al., 2008; Hankinson, 1995). Ligand binding exposes an N-terminal nuclear localization signal and triggers translocation of AHR to the nucleus (Greenlee and Poland, 1979; Ikuta et al., 2000; Okey et al., 1979). Once in the nucleus, AHR binds to aryl hydrocarbon receptor nuclear translocator (ARNT), dissociates from its cytosolic binding partners and binds dioxin-response elements (DREs) in the upstream promoter of AHR-regulated genes (Baba et al., 2001; Denison et al., 1989, 2011; Durrin and Whitlock, 1989; Hankinson, 1995; Quattrochi et al., 1994; Tang et al., 1996). The expression of numerous genes, including xenobiotic metabolizing

Abbreviations: AHR, aryl hydrocarbon receptor; AHR-KO, aryl hydrocarbon receptor knockout; Ahrr, aryl hydrocarbon receptor repressor; AIP, aryl hydrocarbon receptor interacting protein; ALB, serum albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; ARNT, aryl hydrocarbon receptor nuclear translocator; AST, aspartate aminotransferase; BLO, urine occult blood; BUN, blood urea nitrogen; BAS, basophils; BILI, urine bilirubin; CA, calcium; CAOX, calcium oxalate crystals; CBC, complete blood count; CHOL, cholesterol; CL, chloride; CREA, creatinine; CYP1A1, cytochrome P450, family 1, subfamily A, polypeptide 1; CYP1A2, cytochrome P450, family 1, subfamily A, polypeptide 2; CYP1B1, cytochrome P450, family 1, subfamily B, polypeptide 1; DRE, dioxin-response element; EOS, eosinophils; EPI, urine epithelial cells; GGT, γ-glutamyl transpeptidase; GLOB, serum globulin; GLUC, serum glucose; HB, hemoglobin; HBSS, Hank's Balanced Salt Solution; HCT, hematocrit; HSP90, 90-kDa heat shock protein; K, potassium; KET, urine ketones; LD_{50}, median lethal dose; LEUC, leukocytes; LYM, lymphocytes; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MON, monocytes; NA, sodium; NEU, neutrophils; PHOS, phosphorus; PLT, platelets; RBC, red blood cells; SG, urine specific gravity; TBA, total bile acid; TBIL, total bilirubin; TCDD, 2,3,7,8-tetracholorodibenzo-*p*-dioxin; TP, total serum protein; TPHO, triple phosphate crystals; TRIG, triglycerides; UBIL, urine bilinogen; UGLU, urine glucose; ULEUC, urine leukocytes; UPRO, urine protein; URBC, urine red blood cells; UWBC, total white blood cells; WT, wild-type; ZFN, zinc finger nuclease.

* Corresponding author at: The Hamner Institutes for Health Sciences, PO Box 12137, 6 Davis Drive, Research Triangle Park, NC 27709-2137, USA. Fax: +1 919 558 1300.
E-mail address: RThomas@thehamner.org (R.S. Thomas).

0041-008X/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.taap.2013.06.024

enzymes (CYP1A1, CYP1A2, CYP1B1) and a competitive negative feedback inhibitor of AHR (aryl hydrocarbon receptor repressor, Ahrr), are upregulated in response to AHR activation by ligand (DeVito et al., 1994; Korkalainen et al., 2004; Shen et al., 1994; Whitlock et al., 1989). AHR then dissociates from the DRE, is exported from the nucleus, and is targeted for degradation via the ubiquitin–proteasome pathway (Ma and Baldwin, 2002; Pollenz, 2002; Roberts and Whitelaw, 1999; Song and Pollenz, 2002).

Aryl hydrocarbon receptor signaling is activated by a large variety of naturally occurring and man-made ligands, including persistent organic pollutants such as polyhalogenated aromatic hydrocarbons, the most potent of which is 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) (Denison et al., 2011; Nguyen and Bradfield, 2008). Exposure of laboratory rodents to TCDD produces a variety of toxicities including, but not limited to wasting, hepatotoxicity, reproductive toxicity, teratogenicity, neurotoxicity, dermal toxicity, immune suppression, epithelial hyperplasia and tumor promotion (Chopra and Schrenk, 2011; Denison et al., 2011; Kerkvliet, 2009; Linden et al., 2010; Mandal, 2005; Matsumura, 2009; Williamson et al., 2005). AHR-KO mice have been used to demonstrate that some of the toxicities observed in rodents following exposure to AHR agonists (e.g., TCDD, benzo[a]pyrene) require expression of a functional AHR (Fernandez-Salguero et al., 1996; Kerkvliet et al., 2002; Peters et al., 1999; Shimizu et al., 2000; Staples et al., 1998). AHR-KO mice are insensitive to thymic involution, increased liver weight, hepatocellular hypertrophy and hydropic degeneration as well as gene induction following acute exposure to TCDD (Fernandez-Salguero et al., 1996; Staples et al., 1998; Walisser et al., 2005). AHR-KO mice exposed to TCDD during gestation do not develop cleft palate or hydronephrosis as observed in wild-type (WT) mice of the same genetic background (Lin et al., 2001; Peters et al., 1999). These studies provide compelling evidence that AHR activation is the initial key event in these toxicities.

There is also evidence that responses to AHR agonists, at the cellular, tissue and whole organism level, can significantly differ across species. In rodents, reported oral LD_{50} values for TCDD range over 1000-fold (Bickel, 1982; Olson et al., 1980; Schwetz et al., 1973; Vos et al., 1974). In a cross species comparison, the livers of C57BL/6 mice administered TCDD had centrilobular infiltration of inflammatory cells accompanied by centrilobular hepatocellular apoptosis and lipidosis of periportal and midzonal hepatocytes. The livers of Sprague–Dawley rats administered doses of TCDD which result in similar tissue concentrations did not have similar histopathology (Boverhof et al., 2006). Results of transcriptomic analysis were consistent with the respective pathologies and supported divergence of liver tissue response across species (Boverhof et al., 2006; Nault et al., 2013). Similarly, comparative microarray analysis of human and rat hepatocytes (Black et al., 2012), mouse and rat hepatocytes (Carlson et al., 2009) and hepatic cell lines of all three species (Dere et al., 2011) treated with TCDD in vitro demonstrated significant divergence in the global transcriptomic response as well as a noted difference in potency of TCDD to affect the expression of orthologous genes across species. In contrast, little is known regarding differences in the endogenous role of the aryl hydrocarbon receptor across species, such as in the case of tissue development.

While the role of AHR as a mediator of chemical toxicity and regulator of xenobiotic metabolizing enzymes is important, tissue abnormalities observed in three independently generated AHR-KO mouse lines also argue that the AHR plays a critical role in normal tissue development and homeostasis. The three AHR-KO mouse lines were generated on C57BL/6N or C57BL/6J backgrounds by targeted disruption of exons 1 or 2 of the AHR gene (Fernandez-Salguero et al., 1996; Mimura et al., 1997; Schmidt et al., 1996). In all three mouse lines, homozygous AHR-KO mice displayed a slower rate of overall growth as compared to wild-type counterparts. AHR-KO mice were viable and fertile, but did display decreased fertility and fecundity (Abbott et al., 1999; Benedict et al., 2003). AHR-KO mice also had abnormalities in the liver including transient hepatocellular vesiculation and prolonged extramedullary hematopoiesis in neonatal animals (Schmidt et al., 1996). Decreased liver weight as well as mild to moderate portal hypercellularity and fibrosis was observed in adult animals (Fernandez-Salguero et al., 1996; Schmidt et al., 1996). The decreased liver weight in AHR-KO mice has been associated with abnormalities in hepatic vascular development, namely a patent ductus venosus (Harstad et al., 2006; Lahvis et al., 2000). The ductus venosus is a venous shunt which diverts blood away from the liver during fetal development. Normally, this shunt resolves in early postnatal life allowing for complete perfusion of the liver by venous blood (Dzialowski et al., 2011; Momma et al., 1992). In AHR-KO mice, the ductus venosus remains patent throughout the life of the animal, thereby limiting perfusion and nutritive support of the liver (Harstad et al., 2006; Lahvis et al., 2000, 2005). Other vascular abnormalities have also been observed in AHR-KO mice including a persistent hyaloid artery in the eye, altered vascularization of the kidney, and cardiac hypertrophy (Lahvis et al., 2000; Lin et al., 2001; Thackaberry et al., 2002). Male and female AHR-KO mice also have abnormalities in the development and function of the prostate and ovaries, respectively (Benedict et al., 2000, 2003; Lin et al., 2001, 2002). Finally, there is evidence that lack of a functional AHR can impact hematopoietic cell development (Fernandez-Salguero et al., 1996; Lindsey and Papoutsakis, 2011).

Given that there are marked differences in the response of varying rodent species to AHR activation, it is conceivable that marked differences in the endogenous role of AHR in tissue development and homeostasis may also exist. Here, we present the initial characterization of an AHR knockout rat generated on a Sprague–Dawley background and compare gross tissue morphology, tissue histology and serum chemistry markers with a previously established AHR-KO mouse model. Overall, these data demonstrate that AHR may play a distinctly different role in tissue development and homeostasis across rodent species.

Methods

Chemicals. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD, 99.1% purity) solubilized in toluene was purchased from AccuStandard (New Haven, CT).

Generation of AHR-KO rat. Founder animals heterozygous for deletion of the AHR were generated by Sigma Advanced Genetic Engineering (SAGE, St. Louis, MO) on a Harlan Sprague–Dawley outbred background using ZFN technology. ZFN constructs were designed to target exon 2 which contains the DNA binding bHLH motif of the AHR gene. Exon 2 was targeted to facilitate comparisons with a previously developed AHR-KO mouse model (Schmidt et al., 1996). A total of 609 embryos were microinjected, of which 374 were implanted across ten pseudo-pregnant rat dams (n = 27–43 embryos/dam). A total of 60 pups were born. Toe clips from each pup were incubated in QuickExtract™ DNA extraction solution (Epicentre Biotechnology, Madison, WI) at 50 °C for 30 min followed by 65 °C for 10 min and 98 °C for 3 min. A region of exon 2 genomic DNA was then amplified by PCR (95 °C for 30 s, 60 °C for 30 s, 68 °C for 45 s, 35 cycles) using JumpStart™ Taq ReadyMix™ (Sigma-Aldrich, St. Louis, MO). Forward primer: AAGCTGGCTCGCAGTGTAGT and reverse primer: TGCCGA CATACCATCAAAGA. Amplified samples were then incubated in USB® ExoSAP-IT® PCR Product Cleanup Kit (Affymetrix, Inc., Santa Clara, CA) at 37 °C for 1 h followed by 85 °C for 15 min. Samples were then sequenced by ELIM Biopharmaceuticals (Hayward, CA) using standard protocols.

Two pups harbored deletion mutations of 2 and 29 base pairs (bp) in length, respectively, in exon 2 of AHR. Both mutations were predicted to result in a premature stop codon within exon 2. The deletion regions were confined to exon 2 of AHR and did not extend into adjacent intronic sequences. Schematics of the deletion mutations are shown in Fig. A.1.

Download English Version:

https://daneshyari.com/en/article/5846677

Download Persian Version:

https://daneshyari.com/article/5846677

Daneshyari.com