Toxicology and Applied Pharmacology 267 (2013) 209–217

Contents lists available at SciVerse ScienceDirect

Toxicology and Applied Pharmacology

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

Functional deficiency of aryl hydrocarbon receptor augments oxygen toxicity-induced alveolar simplification in newborn mice

Binoy Shivanna *, Wenyan Zhang, Weiwu Jiang, Stephen E. Welty, Xanthi I. Couroucli, Lihua Wang, Bhagavatula Moorthy

Division of Neonatal–Perinatal Medicine, Department of Pediatrics, Texas Children's Hospital, Baylor College of Medicine, Houston, TX 77030, United States

ARTICLE INFO

Article history:
Received 18 October 2012
Revised 31 December 2012
Accepted 2 January 2013
Available online 18 January 2013

Keywords:
Aryl hydrocarbon receptor
Hyperoxia
Alveolar simplification
Lung inflammation

ABSTRACT

Hyperoxia contributes to the development of bronchopulmonary dysplasia (BPD) in premature infants. New BPD is characterized as having alveolar simplification. We reported previously that aryl hydrocarbon receptor (AhR) deficiency increased susceptibility to hyperoxic lung injury in adult mice, and this was associated with decreased expression of cytochrome P450 1A enzymes and increased lung inflammation. Whether AhR protects newborn mice against hyperoxia-induced alveolar simplification is unknown. Thus, we tested the hypothesis that decreased activation of the pulmonary AhR augments hyperoxia-induced alveolar simplification and lung inflammation in newborn mice. Experimental groups included one-day old wild type (WT) and AhR dysfunctional (AhRd) mice exposed to 21% O_2 (air) or 85% O_2 (hyperoxia) for 14 days. Exposure of newborn WT mice to hyperoxia resulted in increased protein, enzyme and mRNA expression of the AhR-regulated lung cytochrome P450 1A1, NAD(P)H quinone oxidoreductase-1, and microsomal glutathione S-transferase 1 enzymes, suggesting that hyperoxia increases activation of the pulmonary AhR. On the other hand, in the AhRd mice, hyperoxia induced the AhR-regulated enzymes to a lesser extent probably due to the dysfunctional AhR in these mice. Alveolar simplification and lung inflammation was increased in mice exposed to hyperoxia compared with those exposed to air, and AhRd mice were more susceptible to hyperoxia-induced alveolar simplification and lung inflammation compared with WT mice. These findings suggest that decreased activation of the pulmonary AhR in newborn AhRd mice augments hyperoxia-induced alveolar simplification and lung inflammation in these mice.

Introduction

Bronchopulmonary dysplasia (BPD) is a chronic lung disease whose primary structural and functional defect is alveolar simplification (Husain et al., 1998; Jobe, 1999). Despite significant improvements in neonatal intensive care management of premature infants, BPD remains the most prevalent complication in these patients affecting approximately 52% of the extremely low birth weight infants (<1000 g birth weight) (Natarajan et al., 2012). Infants developing BPD are more likely to have long-term pulmonary problems, increased re-hospitalizations during the first year of life, and abnormal neurodevelopment compared to infants of similar birth weights and gestation that do not develop BPD (Fanaroff et al., 2007; Short et al., 2003). Thus, there is an urgent need for improved therapies in the prevention and treatment of BPD.

Supplemental oxygen is commonly administered as an important and life-saving measure in patients with impaired lung function. Although delivery of enriched oxygen relieves the immediate life-threatening consequences of hypoxemia, it may also exacerbate lung injury (Thiel et al., 2005). Excessive oxygen exposure and lung stretching leads to increased reactive oxygen species (ROS) production and expression of proinflammatory cytokines (Jobe et al., 2008). ROS react with nearby molecules (e.g., protein, lipids, DNA, and RNA) and modify their structure and function (Bhandari, 2010), and alter signal transduction pathways all of which frequently result in chronic pulmonary toxicity such as BPD. In addition, the antioxidant defense system develops late in gestation, making preterm neonates highly susceptible to oxidative stress (Asikainen and White, 2005; Vina et al., 1995). Evidence implicates oxidative stress (Bhandari, 2010; Saugstad, 2010) and inflammation (Wright and Kirpalani, 2011) as major contributors to the development of BPD and its sequelae. However, the molecular mechanisms by which oxidative stress and inflammation cause BPD remain poorly understood.

The aryl hydrocarbon receptor (AhR) is a member of basic-helix-loop-helix/PER-ARNT-SIM family of transcriptional regulators (Burbach

Abbreviations: BPD, Bronchopulmonary dysplasia; ROS, Reactive oxygen species; AhR, Aryl hydrocarbon receptor; CYP, Cytochrome P450; NQO1, NAD(P)H quinone oxidoreductase-1; MGST1, Microsomal glutathione S-transferase1; WT mice, C57BL6/J wild type mice; AhRd mice, Aryl hydrocarbon receptor dysfunctional mice; RAC, Radial alveolar count; MLI, Mean linear intercept; MCP-1, Monocyte chemoattractant protein-1; ANOVA, Analysis of variance.

* Corresponding author at: Division of Neonatal–Perinatal Medicine, Texas Children's Hospital, Baylor College of Medicine, 1102 Bates Avenue, MC: FC530.01, Houston, TX 77030, United States. Fax: +1 832 825 3204.
E-mail address: shivanna@bcm.edu (B. Shivanna).

0041-008X/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.taap.2013.01.003

et al., 1992). The AhR is expressed in all mouse tissues (Abbott et al., 1995), and in humans, AhR is highly expressed in the lungs, thymus, kidney, and liver (Tirona and Kim, 2005). AhR activation results in the translocation of the cytosolic AhR to the nucleus, where it dimerizes with the AhR nuclear translocator to form a heterodimeric transcription factor. The heterodimeric transcription factor activates the transcription of many phase I and phase II detoxification enzymes such as cytochrome P450 (CYP) 1A1, CYP1A2, glutathione S-transferase-α (GST-α), NAD(P)H quinone oxidoreductase-1 (NQO1), UDP glucuronosyl transferase (UDPGT), and aldehyde dehydrogenase (ALDH), which are encoded by the Ah gene locus (Emi et al., 1996; Favreau and Pickett, 1991; Rushmore et al., 1990). AhR is of particular interest to toxicologists and extensive research has been conducted on its role in the bioactivation of polycyclic and aromatic hydrocarbons leading to carcinogenesis (Nebert et al., 2004). Transgenic mice with AhR deficiencies have provided insight into the potential role(s) that AhR might play in normal physiological homeostasis (Bock and Kohle, 2009; Fujii-Kuriyama and Kawajiri, 2010). We reported earlier that adult mice deficient in AhR are more susceptible to hyperoxic lung injury compared with wild type controls and this phenomenon was associated with marked decreases in the expression of pulmonary and hepatic CYP1A subfamily of enzymes that have been reported to detoxify lipid hydroperoxides generated by reactive oxygen species (ROS) (Couroucli et al., 2002; Jiang et al., 2004). Recently, the AhR has been shown to attenuate tobacco smoke-induced inflammation in the lungs (Baglole et al., 2008; Thatcher et al., 2007), suggesting that AhR is a suppressor of lung inflammation. However, whether AhR attenuates hyperoxia-induced inflammation and alveolar simplification in the newborn lungs are unknown, and the current study was done to address this gap. Hence, the objective of our study was to elucidate the mechanistic role of AhR in hyperoxia-induced alveolar simplification and lung inflammation in newborn mice. We pursued our objective by testing the hypothesis that decreased activation of the pulmonary AhR augments hyperoxia-induced alveolar simplification and lung inflammation in newborn mice.

Materials and methods

Animals. This study was approved and conducted in strict accordance with the federal guidelines for the humane care and use of laboratory animals by the Institutional Animal Care and Use Committee of Baylor College of Medicine (Protocol no: AN-5631). C57BL6/J wild type (WT) and aryl hydrocarbon dysfunctional B6.D2N-Ahr^d/J (AhRd) mice were obtained from Charles River Laboratories (Wilmington) and Jackson Laboratories (Bar Harbor, ME), respectively. Dr. Daniel Nebert (University of Cincinnati, Cincinnati, OH) initially backcrossed Ahr^d allele from DBA/2N onto C57BL/6N via a backcross–intercross breeding scheme and transferred this congenic to Dr. Alan Poland (University of Wisconsin, Madison, WI) at generation N13, who then backcrossed the Ahr^d allele onto C57BL/6J, again via a backcross–intercross breeding scheme. The resulting homozygotes at or beyond generation N17 were maintained at the Jackson Laboratory by sibling intercross. The AhR dysfunction in AhRd mice is due to decreased affinity of the AhR^d receptor for its ligand. There are 10 nucleotide differences in the coding regions between the AhR^b allele present in WT mice and the AhR^d allele present in AhRd mice. The structural changes in the AhR^d receptor associated with these nucleotide differences is thought to be responsible for the differential agonist affinity between the AhR^b and AhR^d receptors (Chang et al., 1993). We maintained active colonies of WT and AhRd mice by breeding them in the animal facility at Texas Children's Hospital's Feigin Center. Time pregnant WT and AhRd mice raised in our animal facility were used for the experiments.

Exposure. Within 12 h of birth, pups from multiple litters were pooled before being randomly and equally redistributed to the dams, following which they were immediately exposed to either 21% O_2 (air) or 85% O_2 (hyperoxia) for 14 days as described earlier (Park et al., 2007). The dams were rotated between air- and hyperoxia-exposed litters every 24 h to prevent oxygen toxicity in the dams and to eliminate maternal effects between the groups. Oxygen exposures were conducted in Plexiglas chambers, into which O_2 was delivered through an oxygen blender to achieve a constant level of 85% O_2. Animals were monitored every 12 h for evidence of adverse lung symptoms or mortality.

Analyses of the pulmonary AhR activation. It is reported that functional activation of the AhR results in the expression of many phase I and II enzymes. So, we determined the functional activation of the pulmonary AhR by analyzing the expression of pulmonary CYP1A1 (phase I), and NQO1 and microsomal glutathione S-transferase 1 (MGST1) (phase II) enzymes.

Lung tissue preparation for analyses of the AhR activation. Following exposure, animals were euthanized with i.p. injections of 200 mg/kg of sodium pentobarbital and their lungs (n = 6/group) were stored at −80 °C for isolation of total RNA. The lungs from a separate set of animals (n = 10/group) were snap frozen in liquid nitrogen for subsequent isolation of nuclear and cytosolic proteins.

Preparation of nuclear and cytosolic protein. A mortar and pestle was used to homogenize the lung tissue in a buffer containing 50 mM Tris–HCL (pH 7.5), 0.5 M KCL, 1 M MgCL, and 0.5 M EDTA. The homogenate was centrifuged at 2400 *g* for 5 min at 4 °C. The supernatant (cytoplasmic fraction) was stored at - 80 °C. The pellet was resuspended in a lysis buffer containing 50 mM Tris-HCL (pH 7.5), 2.1 M NaCL, 1 M MgCL, 0.5 M EDTA, and 25% sucrose, incubated on ice for 20 min, and centrifuged at 19,000 *g* at 4 °C for 5 min. The resulting supernatant (nuclear fraction) was stored at −80 °C until further use.

Enzyme assays. CYP1A1 and NQO1 enzyme activities were measured in the cytosolic fraction according to the published protocols (Benson et al., 1980; Moorthy et al., 2000; Preusch et al., 1991). GST enzyme activity was quantified by using a GST assay kit according to the manufacturer's protocol (Sigma-Aldrich, St. Louis, MO; CS0410).

Western blot assays. Ten or 20 μg of lung cytosolic protein extracts were separated by 10% SDS-polyacrylamide gel electrophoresis for detection of CYP1A1, NQO1, and MGST1 apoproteins, and transferred to polyvinylidene difluoride membranes. The membranes were incubated overnight at 4 °C with the following primary antibodies: anti-CYP1A1 antibody (gift from P.E. Thomas, Rutgers University, Piscataway, NJ, USA; dilution 1:1500), anti-NQO1 antibody (Santa Cruz Biotechnologies; sc-16464, dilution 1:500), anti-MGST1 antibody (Santa Cruz; sc-17003, dilution 1:500) and anti-β-actin antibody (Sigma-Aldrich; A5316, dilution 1:5000). The primary antibodies were detected by incubation with the appropriate horseradish peroxidase-conjugated secondary antibodies. The immunoreactive bands were detected by chemiluminescence methods and the band density was analyzed by Kodak 1D 3.6 imaging software (Eastman Kodak Co., Rochester, NY, USA).

Quantitative real-time RT-PCR assays. Total RNA extracted from frozen lung tissues using Trizol reagent (Invitrogen) were treated with RQ1 RNase-free DNase I (Promega, Madison, WI) to eliminate genomic DNA contamination. RNA (50 ng), isolated as above, was subjected to one-step real-time quantitative TaqMan RT-PCR. Gene specific primers (CYP1A1-Mm00487218_m1; NQO1-Mm01253561_m1; MGST1-Mm00498294_m1; and 18S-Hs99999901_s1) in the presence of TaqMan reverse transcription reagents were used to reverse transcribe RNA, and TaqMan Gene Expression probes and TaqMan Universal PCR Master Mix (Applied Biosystems) were used for PCR amplification. The