Increased susceptibility to hyperoxic lung injury and alveolar simplification in newborn rats by prenatal administration of benzo[a]pyrene

Vijay S. Thakur[a], Yanhong W. Liang[a], Krithika Lingappan[a], Weiwu Jiang[a], Lihua Wang[a], Roberto Barrios[b], Guodong Zhou[c], Bharath Guntupalli[a], Binoy Shivanna[a], Paramahamsa Maturu[a], Stephen E. Welty[a], Bhagavatula Moorthy[a], Xanthi I. Couroucli[a,*]

[a] Department of Pediatrics, Section of Neonatology, Texas Children's Hospital, Baylor College of Medicine, 1102 Bates Avenue, MC: FC530.01, Houston, TX 77030, USA
[b] Department of Pathology and Genomic Medicine, The Methodist Hospital Physician Organization, 6565 Fannin Street, Suite M227, Houston, TX 77030, USA
[c] Institute of Biotechnology, Texas A&M University Health Science Center, Houston, TX 77030, USA

HIGHLIGHTS

- Prenatal benzo[a]pyrene (BP) potentiated hyperoxic lung injury in newborn rats.
- Prenatal BP exacerbated alveolar simplification and inflammation in newborn rats.
- Maternal BP and neonatal hyperoxia significantly modulated CYP1 enzyme expression.
- Oxidative stress contributed to the BP-mediated effects on newborn lung.

ARTICLE INFO

ABSTRACT

Maternal smoking is one of the risk factors for preterm birth and for the development of bronchopulmonary dysplasia (BPD). In this study, we tested the hypothesis that prenatal exposure of rats to benzo[a]pyrene (BP), a component of cigarette smoke, will result in increased susceptibility of newborns to oxygen-mediated lung injury and alveolar simplification, and that cytochrome P450 (CYP)1A and 1B1 enzymes and oxidative stress mechanistically contribute to this phenomenon. Timed pregnant Fisher 344 rats were administered BP (25 mg/kg) or the vehicle corn oil (CO) on gestational days 18, 19 and 20, and newborn rats were either maintained in room air or exposed to hyperoxia (85% O_2) for 7 or 14 days. Hyperoxic newborn rats prenatally exposed to the vehicle CO showed lung injury and alveolar simplification, and inflammation, and these effects were potentiated in rats that were prenatally exposed to BP. Prenatal exposure to BP, followed by hyperoxia, also resulted in significant modulation of hepatic and pulmonary cytochrome P450 (CYP)1A and 1B1 enzymes at PND 7–14. These rats displayed significant oxidative stress in lungs at postnatal day (PND) 14, as evidenced by increased levels of the F_2-isoprostane 8-iso-PGF2α. Furthermore, these animals showed BP-derived DNA adducts and oxidative DNA adducts in the lung. In conclusion, our results show increased susceptibility of newborns to oxygen-mediated lung injury and alveolar simplification following maternal exposure to BP, and our results suggest that modulation of CYP1A/1B1 enzymes, increases in oxidative stress, and BP-DNA adducts contributed to this phenomenon.

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1. Introduction

Maternal smoking is one of the risk factors for preterm birth and intra-uterine growth retardation (Andres and Day, 2000; Delpisheh et al., 2006; Huuskonen et al., 2008; Nieuwenhuijsen et al., 2013; Teune et al., 2012) resulting in serious health problems for the child during the neonatal period, which could in turn lead to chronic lifelong disorders such as cardiovascular disability, cerebral palsy,

mental retardation, and even death (Barker et al., 1995; Horta et al., 1997; Jules et al., 2012). Intrauterine smoke exposure could also influence the development of bronchopulmonary dysplasia (BPD) (Antonucci et al., 2004; Singh et al., 2013). Polycyclic aromatic hydrocarbons (PAHs) such as benzo[a]pyrene (BP) are present in significant quantities in cigarette smoke. Maternal diet is also the main source of in utero exposure to contaminants such as PAHs (Phillips, 1999; Suzuki and Yoshinaga, 2007). PAHs cross the placental barrier, and therefore compromise fetal development (Autrup and Vestergaard, 1996; Sanyal et al., 2007).

BP is known human carcinogen, mutagen, and endocrine disruptor, and has been widely used as a marker for exposure of total carcinogenic PAH (Agency for Toxic Substances and Disease, 1995). Oral exposure to BP is known to induce developmental and reproductive toxicity in experimental studies in animals, including fetal growth (Duarte-Salles et al., 2013). Recent epidemiological studies suggest an association between dietary BP intake and lower birth weight in children (Duarte-Salles et al., 2010, 2013). PAHs, by themselves are inert, but they induce enzymes such as cytochrome P450 (CYP)1A1, 1A2, and 1B1, which are involved in the activation of BP to reactive metabolites that in turn bind to protein and DNA, leading to carcinogenesis (Guengerich, 1988; Smerdova et al., 2013; Xue and Warshawsky, 2005). Maternal exposure to cigarette smoke induces CYP1A1 in placenta as well as fetus, resulting in teratogenic effects in the offspring (Huuskonen et al., 2008; Jedrychowski et al., 2013).

Bronchopulmonary dysplasia (BPD), which is characterized pathologically by diffuse alveolar enlargement, thinning of the septae and narrowing of bronchiolar diameters, also known as chronic lung disease of prematurity is the most common morbidity affecting premature babies with an incidence as high as 52% in extremely low birth weight (birth weights < 1000 g) neonates (Natarajan et al., 2012). It also has long-term consequences such as chronic pulmonary morbidity, increased re-hospitalization rates, development of pulmonary hypertension and delayed neurodevelopment (Ambalavanan et al., 2011; Natarajan et al., 2012; Slaughter et al., 2011). Oxygen toxicity is thought to play a role in both acute lung injury and BPD. Prolonged exposure of newborn mice to hyperoxia, leads to lung pathology similar to human BPD (Warner et al., 1998). In critically ill patients, hyperoxia may exacerbate or even cause acute lung injury. Exposure to hyperoxia postnatally is thought to contribute to the development of BPD in neonates (Vento et al., 2009). Hyperoxia leads to the production of reactive oxygen species (ROS) and these molecules lead to lung injury via oxidation of cellular macromolecules including DNA, protein and lipid (Freeman and Crapo, 1981). The molecular mechanisms by which hyperoxia causes lung injury are not understood, but CYP enzymes have been implicated (Hazinski et al., 1995). On the other hand, studies from our laboratory have demonstrated the protective effect of CYP1A enzymes against hyperoxic lung injury (Couroucli et al., 2011, 2002; Jiang et al., 2004; Moorthy et al., 2000; Sinha et al., 2005). However, there have been no studies on the effect of maternal exposure of environmental PAHs on hyperoxic lung injury in the offspring. Therefore, in this investigation, we tested the hypothesis that prenatal exposure of rats to the PAH BP will result in increased susceptibility of newborns to oxygen-mediated lung injury and alveolar simplification, and that CYP1A and 1B1 enzymes and oxidative stress mechanistically contribute to this phenomenon.

2. Materials and methods

2.1. Animals

Thirteen days pregnant Fisher 344 rats were purchased from Harlan Sprague-Dawley (Indianapolis, IN) and were divided into two groups. Purified tap water and rat chow (Purina Rodent Lab Chow No. 5001 from Purina Mills, Inc., Richmond, Indiana) were made available to animals ad libitum. On days 18, 19 and 20 of pregnancy, one group (experimental) was administered i.p., 25 mg/kg/BW/day (w/v) BP dissolved in corn oil (CO) and another (control group) was administered only CO. Newborn rats delivered from mothers of both these groups were either maintained in room air or placed in oxygen chambers (85% O_2) immediately after birth for up to 7 days (first group) or 14 days (second group). All animal experiments were carried out in accordance to the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health. The experiments performed were reviewed and approved by the Institutional Animal Care and Use Committee of Baylor College of Medicine.

2.2. Hyperoxia exposure

The newborn animals were either maintained in room air or exposed to 85% O_2 for 7 or 14 days whereas dams were rotated between hyperoxic and room air chambers once every 24 h to prevent oxygen toxicity to mothers.

2.3. Chemicals

Buffer components for electrophoresis and Western blotting were obtained from Bio-Rad Laboratories (Hercules, CA). The primary monoclonal antibody to CYP1A1, which cross-reacts with CYP1A2 was a generous gift from Dr. P. E. Thomas (Rutgers University, Piscataway, NJ), CYP1B1 (SC-32882) and β-actin (SC-47778) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA), Goat anti-mouse IgG and Goat anti-rabbit IgG conjugated with horseradish peroxidase were from Bio-Rad laboratories (Richmond, CA). BP (B1760-1G) was purchased from Sigma–Aldrich, Saint Louis MO. Antibody to anti-mouse/anti-rat F4/80 (123102) for immunohistochemical analysis was purchased from BioLegend Inc, San Diago, CA, ECL reagent HyGlo (E5200) was purchased from Denville Scientific, Inc. (Metuchen, NJ) and TRIzol reagent (15596018) was purchased from life Technologies, Carlsbad, CA.

2.4. Tissue perfusion and harvesting

At the completion of exposure to hyperoxia for 7 or 14 days, the animals were sacrificed and their lungs and liver tissue were collected. The left lungs were fixed with zinc formalin by injecting formalin at a constant pressure (20 cm of H_2O) through intratracheal catheter. Lungs were later embedded in paraffin for histological and immunohistochemical analyses. The right lungs were used for RNA isolation for qPCR, tissue lysate preparation for Western blot analysis and F_2-isoprostane estimation. Tissue lysate of livers were also prepared for CYP1A1/1A2 and CYP1B1 analysis and RNA from liver was isolated for qPCR.

2.5. Preparation of tissues for histology

Paraffin embedded tissues were sectioned at 4 μm on a rotary microtome and were stained with hematoxylin and eosin (H&E) and anti-mouse/anti-rat F4/80 antibody. Stained sections were assessed for tissue morphology, injury and inflammation.

2.6. Western blotting

Lung or liver tissue lysate were prepared from the tissues of individual animals in lysis buffer (0.25 M Sucrose, 80 mM Tris–HCl, 5 mM $MgCl_2$, 1 mM EDTA, 1 mM DTT, 1 mM PMSF and protease inhibitor cocktail tablet [(1 tablet in 10 ml), pH 7.4)]. Protein concentrations of the tissue lysate were determined by the Bradford method (Bio-Rad laboratories, Richmond, CA). Equal amount of total proteins were then resolved on SDS–10% polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membrane. The membrane was blocked in 5% nonfat skimmed milk and incubated with primary antibodies specific to CYP1A1, 1A2 or CYP1B1 overnight at 4 °C followed by incubation with secondary antibodies for 1 h at room temperature. Proteins were visualized using ECL reagent as directed by the manufacturer. The densitometry of bands on Western blot analysis was done using image J software and protein to β-actin densitometry ratio was analyzed using 2 way ANOVA followed by Bonferroni post hoc tests to compare each group to all other groups using GraphsPad Prism5 software program. P values less than 0.05 were considered to be statistically significant.

2.7. Real time reverse transcriptase-polymerase chain reaction (RT-PCR) assays

Total RNA from the livers of rats was isolated using Trizol reagent. Sixty nanograms RNA from livers and 60 ng RNA from lungs of air-breathing or hyperoxic animals was subjected to one step real time quantitative TaqMan RTPCR using primers purchased from Applied Biosystems (CYP1A1: Rn00487218-m1; CYP1A2: Rn00561082-m1; CYP1B1: Rn00564055-m1 and 18s: Hs99999901) and detected using ABI PRISM 7700 Sequence Detection System. Each sample was analyzed in duplicate. The relative Ct values for CYP1A1, 1A2 and CYP1B1 were normalized to their 18S content and the relative expression levels of target genes were calculated according to the equation, $2^{-\Delta\Delta Ct}$, where $\Delta\Delta Ct = \Delta Ct$ target gene $- \Delta Ct$ control gene, as reported by us previously (Couroucli et al., 2011).