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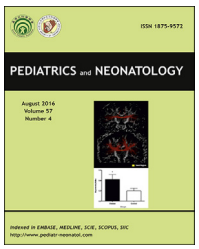

Original Article

Postnatal hyperoxia or DEHP exposure leads to growth restriction and delayed lung development in newborn rats

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Key Words
lung development;
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Background: Di-(2-ethylhexyl) phthalate (DEHP) is commonly used as a plasticizer in many medical devices. We previously showed that maternal DEHP exposure led to restricted growth and delayed lung maturation in newborn rats. As oxygen toxicity continues to be a major risk factor for bronchopulmonary dysplasia, the aim of this study was to examine the effect of hyperoxia, DEHP or DEHP combined with hyperoxia on the growth and lung maturation of newborn rats.
Methods: Newborn rats received DEHP injection, hyperoxia exposure or DEHP injection combined with hyperoxia exposure for one week or two weeks. A control group received an equal volume of vehicle and was maintained in room air.
Results: Hyperoxia and hyperoxia + DEHP exposure for one week led to growth failure in newborn rats. Pups in the hyperoxia group showed catch-up growth after being maintained in room air for an additional 7 days but this was not the case with the latter group, which continued to receive DEHP. Hyperoxia and DEHP both delayed lung development, as evidenced by decreased radial alveolar count. Quantitative RT-PCR showed that hyperoxia decreased the transcripts of VEGF, VEGFR-2 and eNOS on days 7 and 14, and DEHP exposure alone also led to decreased expression of VEGF gene in 14-day-old rat pups.

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Conclusion: Postnatal hyperoxia and/or DEHP exposure lead to growth restriction and delayed lung alveolar development. The VEGF gene expression was altered and may be involved as one of the possible molecular mechanisms.

1. Introduction

Bronchopulmonary dysplasia (BPD) is a chronic lung disease mainly observed in premature infants. It remains one of the most significant causes of morbidity for surviving premature infants, with long-term respiratory and neurodevelopmental consequences that extend beyond the newborn period.[1] BPD is characterized by arrested alveolar development or loss of alveoli, with fewer and dysmorphic capillaries.[2] It is assumed that multiple risk factors, including oxygen toxicity and barotraumas, contribute to the pathogenesis of BPD.[3]

Oxygen toxicity continues to be a major risk factor for BPD despite the fact that we are more cautious with oxygen therapy than ever before. Prolonged exposure to supra-physiologic oxygen concentrations results in increased production of cytotoxic oxygen free radicals that can overwhelm the host antioxidant defense mechanisms[4] and cause lung injury, characterized by the death of alveolar epithelial cells and endothelial cells.[5] In addition to oxygen toxicity and barotraumas, other factors, such as environmental toxins, may also have effects on alveolar development. Due to their immature organ systems, preterm infants might be at an increased risk for adverse health outcomes related to environmental toxins such as di-(2-ethylhexyl) phthalate (DEHP). DEHP is commonly used as a plasticizer in many medical devices made of polyvinyl chloride, such as storage containers, bags and tubes. We previously showed that maternal DEHP exposure at dosages of 0, 10, 100 or 750 mg/kg/day from gestational day 12 to postnatal day 21 led to restricted growth, but only at high dose (750 mg/kg/day) delayed lung maturation in newborn rats.[6] It has been shown that the effects of DEHP are mediated by reactive oxygen species and the apoptosis pathway.[7,8] The highest human exposures to DEHP occur in newborns and infants undergoing extensive medical procedures, such as transfusion, extracorporeal membrane oxygenation, and total parenteral nutrition, through peripherally inserted central catheters. Premature infants have both reduced renal clearance based on decreased glomerular filtration rates and immature glucuronidation in the liver, which can increase the concentration of DEHP metabolites even at low levels of exposure.[9,10] The aim of this study was to examine the effect of hyperoxia, DEHP or DEHP + hyperoxia on body growth and lung development of newborn rats.

2. Materials and methods

2.1. Hyperoxia-induced lung injury

Timed-pregnant Sprague—Dawley rats were purchased from SLAC Laboratory Animal Co. Ltd (Shanghai, China) and maintained in the experimental animal facility of Wenzhou Medical University. All animal procedures were performed in accordance with the policies and guidelines of the Ethics Committee of Wenzhou Medical University. The dams were housed in humidity- and temperature-controlled rooms on a 12:12-h light—dark cycle and were allowed food and water ad libitum. On day 22 of pregnancy, the dams delivered naturally. The pups were pooled, randomized, and returned to the nursing dams. One set of pups was maintained in 85% O_2 for 6 days while the other set was maintained in room air (21% O_2). Nursing dams were rotated between hyperoxia- and room air-exposed litters every 24 h to prevent O_2 toxicity in the nursing dams. Continuous 85% O_2 exposure was achieved in a Plexiglass chamber flow-through system. The O_2 level inside the Plexiglass chamber was monitored continuously with an O_2 analyzer.

Experimentally, the pups were divided into four experimental groups: (1) control group (room air + vehicle, n = 14); (2) DEHP group (room air + DEHP, n = 14); (3) O_2 group (85% O_2 + vehicle, n = 19), and (4) O_2 + DEHP group (85% O_2 + DEHP, n = 17). DEHP (Sigma—Aldrich, St. Louis, MO, USA) was first dissolved in corn oil (Sigma—Aldrich, St. Louis, MO, USA) and administered via intraperitoneal injection (IP) everyday in a volume of 50 μL for a final dose of 750 mg/kg/day. The dose of was according to our previous study.[6] The pups in the room air or O_2 only group were administered the same volume of the vehicle, i.e., corn oil. After 7 days, approximately half of the rats in each group were sacrificed, and their lungs were removed and processed for morphometric, quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) analyses. The remaining half continued to receive either DEHP or vehicle, but they were maintained in room air for additional 7 days. By day 14, the pups were all sacrificed and their lungs were removed and processed for morphometric and quantitative RT-PCR analyses.

2.2. Lung histology and morphometric analyses

Following euthanasia, the pups were assigned for morphometric analysis or for quantitative RT-PCR analysis. For histology and morphometric analyses, after ligation of the right principal bronchus, the left lungs were inflated and fixed with 4% paraformaldehyde in PBS solution through the trachea under a constant pressure maintained by suspending the paraformaldehyde 20 cm above the lab bench for 3 min. The trachea was then ligated, and the left lung was immersed in fixative overnight at 4 °C. The lungs were paraffin-embedded and cut into 4-μm-thick serial sections, and then they were stained with hematoxylin and eosin or immunohistochemistry analysis. Radial alveolar

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