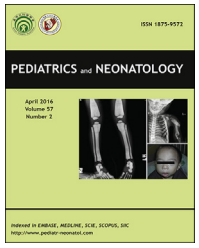
Pediatrics and Neonatology (2016) xx, 1–7

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.pediatr-neonatol.com



ORIGINAL ARTICLE

Uteroplacental Insufficiency Alters the Retinoid Pathway and Lung Development in Newborn Rats

Liang-Ti Huang [a,b], Hsiu-Chu Chou [c], Chun-Mao Lin [d], Chung-Ming Chen [b,e,*]

[a] *Department of Pediatrics, Wan Fang Hospital, Taipei Medical University, Taipei, Taiwan*
[b] *Department of Pediatrics, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan*
[c] *Department of Anatomy and Cell Biology, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan*
[d] *Department of Biochemistry, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan*
[e] *Department of Pediatrics, Taipei Medical University Hospital, Taipei, Taiwan*

Received Dec 16, 2015; received in revised form Feb 21, 2016; accepted Mar 14, 2016
Available online ■ ■ ■

Key Words
alveolarization;
intrauterine growth retardation;
retinoic acid;
uteroplacental insufficiency

Background: Intrauterine growth retardation (IUGR) is associated with reduced lung function during infancy and perhaps throughout adulthood. The retinoic acid (RA) signaling pathway modulates pre- and postnatal lung development. This study was conducted to test our hypothesis that uteroplacental insufficiency alters the elements of the retinoid pathway in developing lungs.

Methods: On Gestation Day 18, either uteroplacental insufficiency was induced through bilateral uterine vessel ligation (IUGR group) or sham surgery (control group) was performed. Lung tissues from the offspring were examined through Western blotting, immunohistochemistry, and morphometry on Postnatal Day 3 and Postnatal Day 7.

Results: Compared with control rats, the IUGR rats exhibited significantly lower body weights on Postnatal Day 3 and Postnatal Day 7 and significantly lower lung weights on Postnatal Day 3. Uteroplacental insufficiency significantly increased RA receptor (RAR)-β protein expression on Postnatal Day 3. The expression of RAR-α, RAR-γ, cellular RA-binding protein-1, and cellular RA-binding protein-2 between the control and IUGR rats was comparable on Postnatal Day 3 and Postnatal Day 7. Compared with the control rats, the IUGR rats exhibited a significantly higher volume fraction of alveolar airspace on Postnatal Day 3 and Postnatal Day 7 and a significantly lower volume fraction of alveolar walls on Postnatal Day 3.

* Corresponding author. Department of Pediatrics, Taipei Medical University Hospital, 252 Wu-Hsing Street, Taipei 110, Taiwan.
E-mail address: cmchen@tmu.edu.tw (C.-M. Chen).

http://dx.doi.org/10.1016/j.pedneo.2016.03.003

Please cite this article in press as: Huang L-T, et al., Uteroplacental Insufficiency Alters the Retinoid Pathway and Lung Development in Newborn Rats, Pediatrics and Neonatology (2016), http://dx.doi.org/10.1016/j.pedneo.2016.03.003

Conclusion: Uteroplacental insufficiency causes defective alveolarization and transient increases in RAR-β expression in the lungs of newborn rats. The retinoid pathway may be one of the probable pathways mediating lung abnormalities caused by uteroplacental insufficiency.

1. Introduction

Recent evidence suggests that adverse environments detrimentally affect the lungs during fetal development, resulting in persistent changes in lung structure and compromised respiratory function during postnatal life.[1] Intrauterine growth retardation (IUGR) is associated with reduced lung function during infancy and perhaps throughout adulthood.[2,3] Alterations in fetal nutritional status may cause developmental adaptations, permanently changing the structure and physiology of offspring, thus predisposing them to pulmonary diseases in adulthood.[4]

Uteroplacental insufficiency—affecting ~10% of human pregnancies—is the most common cause of IUGR.[5] It is characterized by reduced placental function and compromised nutrient and oxygen delivery to the fetus.[6] Similar to humans, uteroplacental insufficiency caused by bilateral uterine vessel ligation in pregnant rats can lead to IUGR in rat fetuses.[7] The mechanisms of lung abnormalities among children born with IUGR require further clarification.

Retinoic acid (RA) is produced during vitamin A metabolism, which involves successive oxidative reactions of dietary precursors including retinyl esters and carotenoids.[8] Morphological studies have demonstrated that RA signaling is essential for lung development.[9] The cells of developing embryos obtain RA through the blood, where it circulates as retinol bound to an RA-binding protein. In the cytoplasm, retinol binds to cellular RA-binding proteins (CRABPs), which facilitate its enzymatic conversion to RA. RA enters the cell nucleus and activates transcription by binding to two classes of transcription factors—RA receptors (RARs) and retinoic X receptors. Retinoids are crucial mediators of prenatal and postnatal lung development.[10–12] A recent animal study demonstrated that alveolar formation and regeneration can be rescued using postnatal RA treatment in calorie-restricted developing rat lungs.[13] In a human study, vitamin A supplementation reduced the incidence of bronchopulmonary dysplasia in extremely low-birth weight infants.[14] Thus, we hypothesized that uteroplacental insufficiency alters the elements of the RA signaling pathway in developing lungs.

2. Materials and methods

2.1. Animal model

This study was performed in accordance with the guidelines provided by the Animal Care Use Committee of Taipei Medical University, Taipei, Taiwan. Time-dated pregnant Sprague—Dawley rats were housed in individual cages with a 12:12-hour light—dark cycle and *ad libitum* access to laboratory food and water. On Gestation Day 18, either uteroplacental insufficiency was induced through bilateral uterine vessel ligation (IUGR group) or sham surgery was performed (control group). All rats were delivered naturally at term (22 days). Litters were separated from their mothers within 12 hours of birth, pooled, and randomly redistributed to the newly delivered mothers. Lung development occurs over the embryonic, pseudoglandular, canalicular, saccular, and alveolar stages; rats are born at the saccular stage, which is equivalent to an approximately 30-week human gestation.[15] Murine alveolar development begins on Postnatal Day 4, which is equivalent to the human alveolar stage. On Postnatal Day 3 and Postnatal Day 7, rats were randomly selected for examination from each group, irrespective of sex.

2.2. Histological analysis

Lung morphometry, examined using point counting through light microscopy, has indicated that a proportion of the parenchyma is highly constant in all lobes.[16] The right lung lobes were sectioned at 5 μm and stained with hematoxylin and eosin, and digitized images of these lung sections were captured in 20 nonoverlapping fields. Images were printed and examined at a final magnification of 400×.[17] The number of points along the alveolar airspace and alveolar walls were counted by superimposing 49-point transparent grids onto enlarged printed images. The alveoli were defined as structures opening into alveolar ducts transversely and enclosed by the respiratory epithelium cross-sectionally. The volume fraction was calculated as Pi/Pt, where Pi indicates the number of test points hitting the structure of interest and Pt indicates the total number of points hitting the reference space.

2.3. Western blotting

Lung tissues were homogenized in 1 mL of ice-cold lysis buffer and centrifuged at 13,000 g for 20 minutes at 4°C; the supernatant was then aliquoted and stored at −20°C. After blocking with 5% nonfat skim milk, the membranes were incubated with polyclonal RAR-α (C-20), RAR-β (C-19), and RAR-γ (G-1) antibodies (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA, USA), and CRABP-1 (ab2816) and CRABP-2 (ab74365) antibodies (1:1000; Abcam, Cambridge, MA, USA); β-actin rabbit monoclonal antibody (1:5000; Cell Signaling Technology, Danvers, MA, USA) was used as an internal control. As secondary antibodies, goat antimouse (#31320) and goat antirabbit (#31460) immunoglobulin G—horseradish peroxidase antibodies (1:10000; Pierce Biotechnology Inc., Rockford, IL, USA) were used. The densitometry unit of protein expression in the control group was assigned as 1 after normalizing with β-actin.

Please cite this article in press as: Huang L-T, et al., Uteroplacental Insufficiency Alters the Retinoid Pathway and Lung Development in Newborn Rats, Pediatrics and Neonatology (2016), http://dx.doi.org/10.1016/j.pedneo.2016.03.003