BASIC INVESTIGATION

The 50/10 Oxygen-Induced Retinopathy Model Serves as a Hyperoxia and Hypoxia Model of Bronchopulmonary Dysplasia

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ABSTRACT

Background: Animal models of bronchopulmonary dysplasia (BPD) are mainly created by hyperoxia exposure. However, these models do not fully recapitulate BPD pathophysiology as observed in clinical practice. To find a better BPD model, we established a rat 50/10 oxygen-induced retinopathy (OIR) model and analyzed the pathologic features of the lungs.

Methods: The rat OIR model was established by exposing newborn rats (P0) to 50% and 10% oxygen (hyperoxia and hypoxia) on alternating days for 14 days. Lungs were harvested immediately on postnatal day 14 (P14) and on P18 after 4 days of normoxia exposure for hematoxylin and eosin staining, antialpha smooth muscle actin (α-SMA) immunohistochemistry and Picrosirius red staining of collagen. Retinas were obtained to confirm successful model establishment by isolectin B4 staining of retinal vasculature.

Results: OIR rats presented with fewer and enlarged alveoli, and the septal walls were thicker than those in age-matched controls. α-SMA immunohistochemistry indicated increased abundance of myofibroblasts in OIR rats. At P18, α-SMA-positive myofibroblasts were present at extremely low levels from the alveolar walls of control rats, while OIR rats showed myofibroblast persistence. The amount of collagen in OIR rats was also higher than that in control rats at both P14 and P18 as evidenced by Picrosirius red staining.

Conclusions: Alveolar changes observed by hematoxylin and eosin staining, prolonged and stronger α-SMA expression and augmented collagen accumulation resemble the histopathology of BPD, suggesting that the rat 50/10 OIR model is suitable for use in BPD research.

Key Indexing Terms: Bronchopulmonary dysplasia; Oxygen-induced retinopathy; Alveolar simplification; Myofibroblast; Collagen. **[Am J Med Sci 2018;▮(▮):▮▮▮–▮▮▮.]**

INTRODUCTION

Bronchopulmonary dysplasia (BPD), also referred to as chronic lung disease in infants, affects approximately 50% of infants born at less than 28 weeks of gestational age.[1] The "old" BPD was most frequently characterized by histologic changes such as bronchial fibrosis and vascular smooth muscle hypertrophy which were caused by invasive ventilation and intensive oxygen therapy. With the advent of antenatal steroids, surfactants, noninvasive ventilation, and improved management of oxygen therapy, an increasing number of very low birth weight and extremely low birth weight infants survive. These infants exhibit distinct histopathologic sequela, with disruption of the alveoli and vasculature as the main characteristics, namely the "new" BPD.[2]

Current BPD models do not accurately recapitulate the pathophysiology of BPD. For instance, an oxygen level higher than 65% is not recommended according to the latest guidelines for neonatal respiratory support,[2,3] but 90% oxygen is used in most hyperoxia-induced BPD models.[3,4] In addition to hyperoxia, premature birth is associated with transient hypoxia and prolonged instability of oxygen concentrations, resulting in BPD, retinopathy of prematurity (ROP), etc.[1] Premature infants may suffer from hypoxia due to respiratory distress syndrome, patent ductus arteriosus, apnea, septic shock and other stressors. However, BPD models may not include transient hypoxia. The analysis of blood gas changes in a 50/10 oxygen-induced retinopathy [OIR; which includes both hyperoxia (50%) and hypoxia (10%)] rat model found that the lowest PaO2 (5 kPa/35 mm Hg) in 10% oxygen and highest PaO2 (25-30 kPa/185-225 mm Hg) in 50% oxygen were similar to measurements in premature babies.[3] Thus, 50/10 OIR may be a

more physiologically relevant model of BPD than conventional hyperoxia induction models. Studies on ROP have established greatly improved animal models with greater relevance to clinical practice. The rat 50/10 OIR model is gradually replacing the mouse hyperoxia model (90% oxygen, postnatal day [P] 7-12).[5]

The present study aimed to explore the feasibility of the rat OIR model in BPD research. We hypothesized that the pathologic characteristics of BPD were present in the hyperoxia and hypoxia-exposed rat pups, and that the OIR model recapitulates many aspects of the histopathology of BPD.

MATERIALS AND METHODS

Establishment of the Rat 50/10 OIR Model and Sample Collection

All animal studies complied with the Guide for the Care and Use of Laboratory Animals, Southeast University, China, and the study was approved by the Research Ethics Committee of Southeast University. Sprague Dawley (SD) rats were purchased from the Animal Core Facility of Nanjing Medical University (Nanjing, China) for the OIR model. Day 1 of pregnancy was confirmed by the presence of spermatozoa in vaginal smears. Pregnant rats were individually housed with free access to food and water under a 12 hours/12 hours light or dark cycle. All dams littered spontaneously, and the day of birth was defined as postnatal day 0 (P0). Shortly after birth, litters and dams were placed in an oxygen-controlled environment alternating between 50% and 10% every 24 hours for 14 consecutive days. At P14, the pups were used for experiments or returned to normoxia for another 4 days (Figure 1).[3,4] At P14 and P18, OIR pups and matched controls were humanely sacrificed by an intraperitoneal injection of 0.06 mg/g ketamine and 0.018 mg/g xylazine, and their eyes and lungs were harvested for further examination.

Retina Preparation for Flat Mounting and Isolectin B4 Staining

Harvested eyes were enucleated, and the surrounding tissue was removed. Enucleated eyeballs were fixed and dehydrated by sequential incubation in 4% paraformaldehyde (PFA) and ×2 phosphate buffered saline (PBS). The retina was obtained by cutting at the junction of the iris and sclera and discarding the anterior vitreous and posterior sclera. The retina was then cut to create a "petal" shape for flat mounting.

Retinas were then incubated in cold methanol (–20°C) to facilitate permeabilization. Isolectin B4 (Sigma-Aldrich, St. Louis) staining was conducted according to manufacturer's protocol. Briefly, retinas were rinsed in PBS, blocked by incubation in perm or block solution (PBS + 0.3% Triton + 0.2% BSA + 5% serum), incubated in isolectin B4 overnight at 4°C, washed 4 times (15 minutes per wash) in PBXTX (PBS + 0.3% Triton), and then mounted on slides with antifade mounting medium (Beyotime, Nanjing, China).[5] Retinas were photographed under a Nikon Eclipse Ci microscope equipped with a ×10/4 objective (Nikon Instruments Inc., Melville, NY). Images were digitized and stored for analysis. The areas of avascular and neovascular regions were calculated using Photoshop CC 2017 (Adobe Systems, San Jose, CA). Images were assembled into montages using Adobe Illustrator 7.0 (Adobe Systems, San Jose, CA) while preserving original image dimensions.

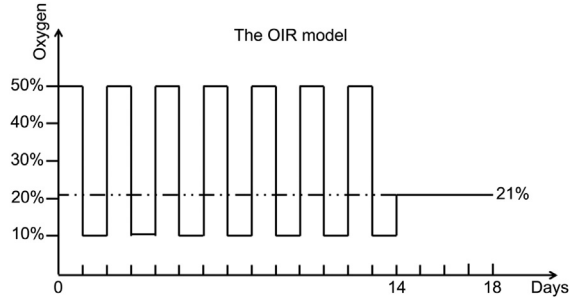

FIGURE 1. The oxygen-induced retinopathy (OIR) model was successfully established. Newborn rats were exposed to cyclical hyperoxia and hypoxia (50% and 10% oxygen every other day) from P0 to P14. Some rats were then subjected to normoxia from P14 to P18.

Lung Fixation and Histologic Examinations

For *in vivo* fixation of lung tissue, treated (OIR group) and age-matched control rats were anesthetized and perfused with 4% PFA in the left ventricle. Then, PFA was directly perfused in the lung. Briefly, the trachea was exposed, and a needle tip connected to a PFA reservoir was inserted. Afterwards, the lungs were perfused for 20 minutes under pressure (25 cm for P14 rats or 35 cm for P18 rats). Next, the left lobe of the lung was excised and fixed with 4% PFA for 24 hours before paraffin embedding. Histologic sections (5 μm) were stained with hematoxylin and eosin (HE), antialpha smooth muscle actin (α-SMA) (ab5694, Abcam, Cambridge, MA) for the detection of myofibroblasts, and Sirius red (365548, Sigma-Aldrich, Poole, UK) for the detection of collagen.

HE Staining and Analysis

Randomly chosen alveolar areas from the OIR and control groups were photographed with ×10/10 and ×10/40 microscope objectives. Fields with large vessels and conducting airways were avoided. The mean linear intercept (MLI), mean alveolar number (MAN), nodal density and septal thickness of each image were calculated in ×10/40 fields and presented in the graph format using GraphPad Prism 7 (GraphPad software INC, San Diego, CA). For the MLI, cross lines were drawn on the center of the picture and the number of times that the lines crossed an alveolar interface was counted as NS. The total length of the cross lines was recorded as L, and the MLI was calculated as L divided by NS. For calculating the MAN, the number of alveoli was counted