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The role of oxygen tension in the regulation of embryonic lung development

Toko Shinkai, Masato Shinkai, Martina A. Pirker, Sandra Montedonico, Prem Puri*

Children's Research Centre, Our Lady's Hospital for Sick Children, University College, Dublin 12, Ireland

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Abstract

Background/Purpose: Oxygen tension is an important physiologic mediator of embryonic and fetal development. In vitro studies have demonstrated that the proper embryonic development is dependent upon low oxygen tension and even short exposure to normoxic environments (21%) can be detrimental to embryonic development. We hypothesized that low oxygen tension promotes lung growth in embryonic organ culture and therefore designed this study to investigate embryonic lung growth in normoxic and hypoxic conditions using simple closed chamber.

Methods: Fetal rat lungs were harvested on day 13.5 and placed in organ culture containing serum-free Dulbecco's modified Eagle's medium with antibiotics. The lung cultures were divided into normoxic group, with a 21% oxygen concentration (n = 15), and hypoxic group (n = 15). Hypoxic condition (6% oxygen) was achieved using Oxoid Campygen in a closed chamber. The lungs were placed in 5% carbon dioxide, 37°C incubator for 48 hours. Media were not changed during the incubation period. The morphometric analysis was measured at 0 hour and at 48 hours by counting total terminal buds and entire epithelial contour using Image J software. The fold increase in branching was calculated as the ratio of buds present at 48 hours minus the buds present at 0 hour divided by the number of buds at 0 hour. The increase in entire epithelial contour over 48 hours was calculated in exactly the same way as described above.

Results: There was no significant difference in the increase in total terminal buds count in the hypoxic group (2.06 ± 0.19) compared with the normoxic group (2.59 ± 0.21), and no significant difference in the increase in entire epithelial contour in the hypoxic group (1.45 ± 0.11) compared with the normoxic group (1.63 ± 0.11).

Conclusions: Although hypoxia has been reported to be an important regulator of murine vascular development, our data show that the embryonic lung growth in whole lung organ culture under hypoxic condition is not significantly different from that in normoxic condition.

In mammalian fetuses and neonates, they have well-developed adaptive mechanisms for surviving prolonged and severe hypoxia. Low oxygen tension is a persistent and dominant feature of the developing environment throughout their gestation [1]. In vitro studies demonstrated that the low-oxygen environment is important during organogenesis, the optimum oxygen concentration for mammalian embryos is considered to be 3% to 5% as the physiologic hypoxia [2-4]. Low-oxygen concentration

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* Corresponding author. Tel.: +353 1 4096420; fax: +353 1 4550201.
E-mail address: prem.puri@ucd.ie (P. Puri).

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is essential in the development of zygotes into blastocysts [5]. Even short exposure to normoxic environments (21% oxygen) can be detrimental to development of blastocyst [2]. In vitro study using embryonic heart and kidney culture showed that vasculogenesis, angiogenesis, and tubulogenesis are stimulated in low-oxygen conditions (3%-5%) [2-4,6]. However, there is little information on the role of hypoxia in the regulation of embryonic lung development. Although many researches on embryonic lung growth in vitro are done using the lung explants cultured at 5% carbon dioxide and 21% oxygen tension, this high oxygen tension can be toxic to the embryonic lung tissue and cause negative effects on the lung growth. Recently, Gebb and colleagues [3,4] demonstrated that fetal lung explants showed increases in terminal branching and cellular proliferation at 3% oxygen in culture. We hypothesized that low oxygen tension promotes lung growth in embryonic organ culture and therefore designed this study to investigate embryonic lung growth in normoxic and hypoxic conditions using simple closed chamber.

1. Materials and methods

1.1. Animals

Timed-pregnant Sprague-Dawley rats, gestational day 13.5 (based on the appearance of vaginal plug, day 0.5), were euthanized with cervical dislocation. Under sterile condition, the fetuses were removed and washed in cold Hank's balanced salt solution. Using a stereomicroscope, the lungs were dissected and transferred into Dulbecco's modified Eagle's medium (DMEM) (GibcoBRL, Life Technologies, UK).

1.2. Organ culture

The lungs were placed on translucent polycarbonate culture dish inserts (3.0 μm pore size, 12 mm diameter; Transwell, Costar, UK) and cultured with serum-free DMEM supplemented with 100 U/mL penicillin and 100 μg/mL streptomycin [7,8]. The lung cultures were divided into normoxic group (n = 15) and hypoxic group (n = 15). The normoxic group was incubated at 37°C, 21% oxygen, and 5% carbon dioxide atmosphere. Hypoxic condition was achieved using Oxoid Campygen (Oxoid Ltd, Hampshire, UK) in a closed chamber. Oxoid Campygen will absorb oxygen and produce carbon dioxide and then the oxygen and carbon dioxide concentration will be around 6% and 10% to 15% in the closed chamber, respectively [9]. The lungs of the hypoxic group were placed in this chamber. This closed chamber was incubated at 37°C, 21% oxygen, and 5% carbon dioxide. Organ cultures were maintained 48 hours. Media were not changed during incubation period. All protocols were carried out in compliance with current European Union regulations for animal investigations

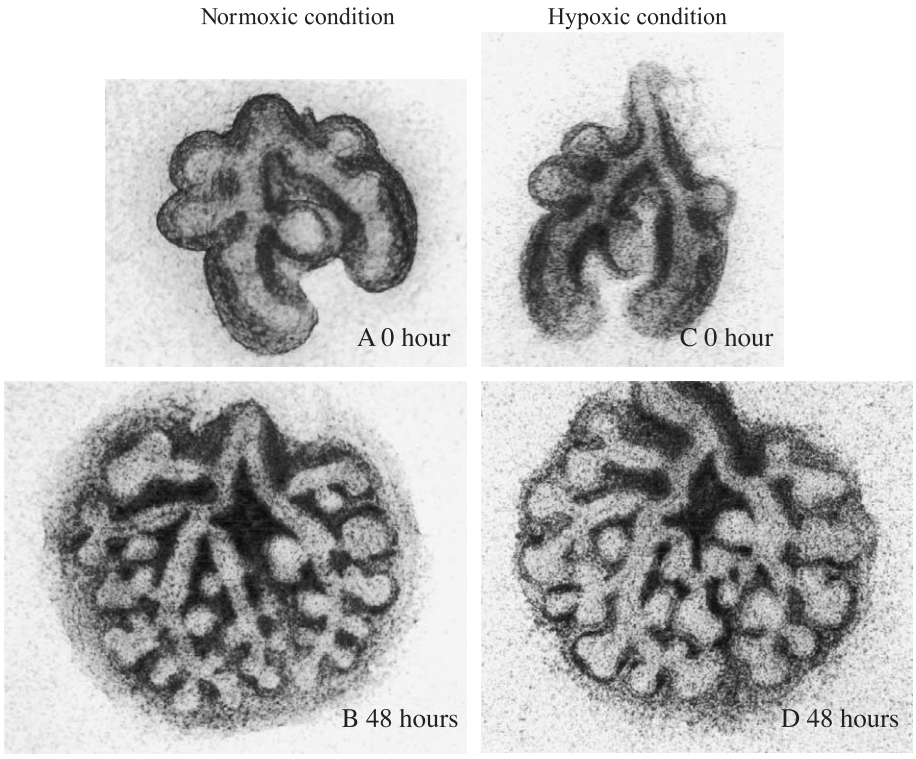

Fig. 1 Representative photographs of cultured embryonic rat lungs. Normoxic condition; lung at 0 hour (A) and after 48 hours (B). Hypoxic condition; lung at 0 hour (C) and after 48 hours (D). Their branching patterns after 48 hours are similar in both normoxic and hypoxic groups. The lungs exhibited the normal progression of monochotomous and dichotomous airway branching in both groups.

(B100/3439) and prior ethical approval under license from the Department of Health, Ireland.

1.3. Morphometric analysis

The morphometric analysis was measured at 0 hour and at 48 hours by counting total terminal buds and entire epithelial contour using Image J software (National Institutes of Health, Bethesda, MD). The fold increase in branching was calculated as the ratio of buds present at 48 hours minus the buds present at 0 hour divided by the number of buds at 0 hour [10]. The increase in entire epithelial contour over 48 hours was calculated in exactly the same way as described above.

1.4. Statistical analysis

Data were presented as the means ± SD. Differences between groups were analyzed by Student's unpaired *t* tests using a commercial software package (StatView, SAS Institute Inc, North Carolina, USA). Significance was set at $P < .05$.

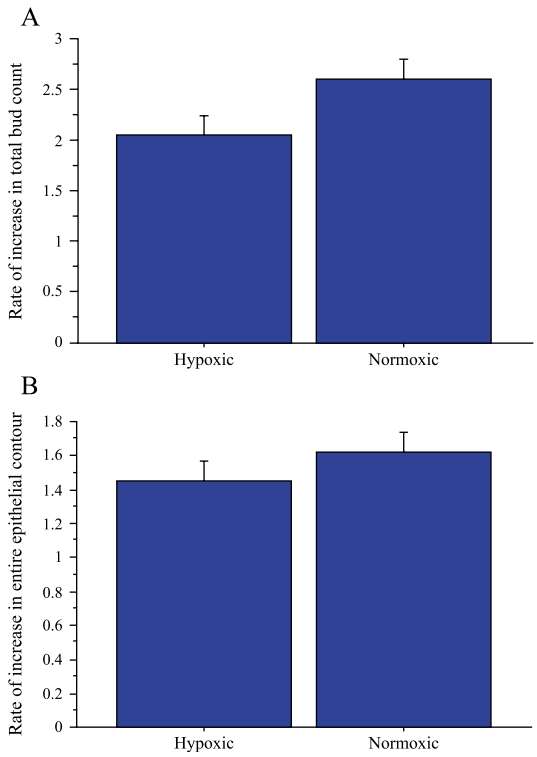

Fig. 2 Morphologic development of normal gestational day 13.5 rat lungs cultured in normoxic and hypoxic conditions. Graphs demonstrated ratio of total terminal bud count (A) and ratio of entire epithelial contour (B). There were no significant differences between normoxic and hypoxic groups.

2. Results

Representative lungs at 0 hour and after 48 hours of culture are shown in Fig. 1. The lungs exhibited the normal progression of monochotomous and dichotomous airway branching in both groups [11,12]. The lungs of the hypoxic group had similar branching pattern with the normoxic group. There was no significant difference in the rate of increase in the total terminal bud count in the hypoxic group (2.06 ± 0.19) compared with normoxic group (2.59 ± 0.21) (Fig. 2A). There was also no significant difference in the rate of increase in the entire epithelial contour in hypoxic group (1.45 ± 0.11) compared with the normoxic group (1.63 ± 0.11) (Fig. 2B). In addition, the peak increase rate of the terminal bud count and the entire epithelial contour in the hypoxic group was slightly lower than that of the normoxic group.

3. Discussion

Recent studies have revealed that the later phase of lung morphogenesis is controlled by region-specific signaling system along the proximodistal axis and signaling cross talk between the endoderm and mesoderm, which are mediated by several transcription factors, for example, Foxf1 and Gli proteins, and secreted proteins, for example, fibroblast growth factors, bone morphogenetic protein 4, and sonic hedge hog [13]. The relationship between the role of hypoxia and these signaling system mentioned above in the embryonic lung morphogenesis is not fully understood.

Oxygen tension is an important physiologic factor of embryonic and fetal development and hypoxia is known to be an important regulator of both vasculogenesis and angiogenesis. However, there is little information on the role of hypoxia in the regulation of embryonic lung development. Fetal lung explants maintained in culture are a well-characterized model for studying lung development, but most of them are cultured at 21% oxygen tension, which is extremely supraphysiologic. We hypothesized that the oxygen tension may negatively interfere with the fetal lung growth. To evaluate the effect of low oxygen tension on fetal lung branching morphogenesis we used a closed chamber containing an oxygen-adsorbent Oxoid Campygen. This system does not need an oxygen tension–controllable incubator and easily achieves hypoxic environment of 6% oxygen tension, which is extremely lower than standard culture condition although slightly higher compared with normal embryonic environment. During the incubation there was no difference in color of the culture medium between the normoxic and hypoxic groups, thus suggesting that the high carbon dioxide concentration did not affect the pH of the culture medium. We confirmed, therefore, that the hypoxic condition did not have negative effect on the lung growth, and, contrary to the previous reports by Gebb

et al [3,4], we did not find significant acceleration of branching in the hypoxic lung compared with the normoxic lung. Because there is a small difference in oxygen tension and gestational age of the fetal lung between the study of Gebb and ours (3% vs 6%, 13.5 vs 15 days, respectively), we assume that the optimum oxygen concentration for promoting fetal lung development may be in a very limited range and its effect may also be variable depending on the gestational age. Further studies using various gas phase, lung stage, and culture period are required to determine the exact role of oxygen tension in the regulation of embryonic lung development.

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