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Alterations of peroxisome proliferator-activated receptor γ and monocyte chemoattractant protein 1 gene expression in the nitrofen-induced hypoplastic lung

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Abstract

Background/Purpose: Peroxisome proliferator-activated receptor γ (PPARγ) plays a key role in normal lung development. Peroxisome proliferator-activated receptor γ messenger RNA (mRNA) is detectable at 18 days of gestation in fetal rat lungs, and levels peak just before birth. Peroxisome proliferator-activated receptor γ agonists are reported to stimulate lung development, whereas inhibition of PPARγ disrupts postnatal lung maturation. Monocyte chemoattractant protein 1 (MCP-1), which is inhibited by PPARγ, is reported to disrupt late lung morphogenesis. This study was designed to investigate the hypothesis that PPARγ expression is downregulated and that MCP-1 expression is upregulated during the late stages of lung development in nitrofen-induced hypoplastic lungs.

Methods: Pregnant rats were treated with nitrofen or vehicle on D9. RNA was extracted from fetal lungs (D18 and D21), and relative mRNA expression levels of PPARγ and MCP-1 were determined by reverse transcriptase–polymerase chain reaction. Immunohistochemistry was performed to evaluate protein expression/distribution of PPARγ and MCP-1.

Results: Relative mRNA expression levels of PPARγ were significantly downregulated in the nitrofen group compared with controls on D21, whereas MCP-1 levels were upregulated. Immunohistochemical study showed markedly decreased PPARγ and increased MCP-1 immunoreactivity in the nitrofen-induced hypoplastic lungs compared with controls on gestational day 21.

Conclusion: Altered pulmonary gene expression of PPARγ and MCP-1 during late gestation may impair lung development and maturation, contributing to pulmonary hypoplasia in the nitrofen-induced congenital diaphragmatic hernia model.

Pulmonary hypoplasia (PH) and persistent pulmonary hypertension remain the main causes of morbidity and mortality in congenital diaphragmatic hernia (CDH) [1]. Various experimental models have been used to study the pathogenesis of PH in CDH [2]. The nitrofen-induced CDH

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model has been widely used to investigate the pathogenesis of PH because of the striking similarities to the human condition [3-5]. It is therefore one of the most accepted animal models to study the pathogenesis of PH in CDH. Because of retarded differentiation of type 2 alveolar epithelial cells (AECs II), disruption in late lung development and alveolar maturation has been reported to contribute to PH in the nitrofen-induced CDH model [6]. Although several studies have recently provided new insights into the pathogenesis of PH associated with CDH, the exact molecular mechanisms underlying nitrofen-induced PH still remain unclear [7-10].

The transcription factor peroxisome proliferator-activated receptor γ (PPARγ) is involved in various cellular functions, such as differentiation and cellular survival [11]. PPARγ is expressed in a broad range of developing organs, including the lung (airway epithelium, bronchial smooth muscle, endothelial cells, macrophages, eosinophils, and dendritic cells) [12]. PPARγ messenger RNA (mRNA) expression is detectable at 18 days of gestation in fetal rat lungs, and it increases during development, peaking just before birth [13].

PPARγ has been reported to play a key role in lung morphogenesis by stimulating alveolar epithelial-mesenchymal paracrine signaling [14]. It has been shown that PPARγ has a significant influence on cellular differentiation, controlling alveolar maturation [12]. Systemically administered PPARγ agonists, such as rosiglitazone, significantly enhance the late stage of lung maturation [14], whereas conditional knockout of PPARγ disrupts alveolar maturation [11].

The chemokine monocyte chemoattractant protein 1 (MCP-1) is the main monocyte chemoattractant that is produced by a variety of cells, including airway epithelial cells, and airway smooth muscle cells on stimulation with cytokines and bacterial and viral products as well as mitogens [15]. Monocyte chemotactic protein 1 has been reported to be inhibited by activation of PPARγ [12,16,17]. In the late stages of lung development, increased pulmonary and circulating MCP-1 levels have been associated with the development of PH in animal models as well as human studies [18-21].

We designed this study to investigate the hypothesis that PPARγ expression is downregulated and that MCP-1 expression is upregulated during the late stages of fetal lung development in the nitrofen-induced CDH model.

1. Materials and methods

1.1. Animals and drugs

Adult Sprague-Dawley rats were mated, and women were checked for plugging. The observation of spermatozoids in the vaginal smear was considered as proof of pregnancy, and this day of observation was determined as gestational day (D) 0. Pregnant female rats were then randomly divided into 2 groups ("nitrofen" and "control"). Under short anesthesia, animals in the nitrofen group (n = 16 at each time-point) received 100 mg of nitrofen intragastrically (WAKO Chemicals, Osaka, Japan), dissolved in 1 mL of olive oil at D9. Animals in the control group (n = 8 at each time-point) received only vehicle. On D18 and D21, cesarean section was performed, and fetal lungs were harvested. All animal experiments were carried out according to the guidelines of animal care. The experimental protocol was approved by the Department of Health and Children (reference B100/4178) under the Cruelty to Animals Act 1876, as amended by European Communities Regulations 2002 and 2005.

1.2. RNA isolation and real-time reverse transcriptase–polymerase chain reaction

From each fetal lung, total RNA was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. Spectrophotometrical quantification of total RNA was performed (NanoDrop ND-1000 UV-Vis Spectrophotometer, Wilmington, USA). Reverse transcription of 1-μg total RNA was carried out using Transcriptor High Fidelity cDNA Synthesis Kit (Roche Diagnostics, West Sussex, United Kingdom) according to the manufacturer's instructions. The resulting complementary DNA was used for quantitative polymerase chain reaction using a LightCycler 480 SYBR Green I Master (Roche Diagnostics) according to the previously described protocol [9]. Gene-specific primer pairs are listed in Table 1. Relative levels of gene expression were determined using a

Table 1 Primer sequences

Gene	Primer sequence	Product size (bp)
PPARγ forward	GACCACTCCCATTCCTTTGA	108
PPARγ reverse	AACCATTGGGTCAGCTCTTG	
MCP-1 forward	CCAGAAACCAGCCAACTCTC	70
MCP-1 reverse	GCGTGACAGAGACCTGCATA	

bp indicates base pair.