Journal of Pediatric Surgery (2009) **44**, 321–324

ELSEVIER

Journal of Pediatric Surgery

www.elsevier.com/locate/jpedsurg

Up-regulation of COUP-TFII gene expression in the nitrofen-induced hypoplastic lung

Takashi Doi, Kaoru Sugimoto, Prem Puri*

The Children's Research Centre, Our Lady's Children's Hospital, Dublin, Ireland
University College Dublin, Dublin, Ireland

Received 3 October 2008; accepted 23 October 2008

Key words:
COUP-TFII;
Retinoid signaling pathway;
Hypoplastic lung;
Congenital diaphragmatic hernia;
Nitrofen

Abstract

Purpose: Recent studies have suggested that the retinoid signaling pathway (RSP) is inhibited in the nitrofen-induced hypoplastic lung. The exact mechanism by which nitrofen acts in the RSP remains unclear. Targeted ablation of COUP-TFII, a gene encoding a transfactor regulated by the RSP, has been shown to cause Bochdalek-type congenital diaphragmatic hernia. It has been shown that COUP-TFII has 2 main roles in the RSP, (i) repressing the RSP by directly sequestering retinoid X receptors, thereby preventing heterodimerization to retinoid acid receptors and inhibiting gene transcription, and (ii) modulating the transcriptional activity of GATA proteins. We designed this study to investigate the gene expression of COUP-TFII in the nitrofen-induced hypoplastic lung.

Materials and Methods: Pregnant rats were exposed to either olive oil or 100 mg of nitrofen on day 9 of gestation. Fetuses were harvested and lungs were dissected on day 15 (D15), D18, and D21 and divided into 2 groups: control (n = 9) and nitrofen (n = 9). Real-time reverse transcription–polymerase chain reaction was performed to evaluate the relative mRNA levels of COUP-TFII expression in the hypoplastic lung.

Results: The relative mRNA levels of COUP-TFII at D15 was significantly increased in the nitrofen group (0.76 ± 0.53) compared to controls (0.45 ± 0.05) ($P < .01$). The expression levels of COUP-TFII at D18 and D21 were not significantly different between the nitrofen group and controls.

Conclusions: Our results provide evidence for the first time that the pulmonary gene expression of COUP-TFII is up-regulated in the early stages of lung development in the nitrofen-induced hypoplastic lung. We speculate that up-regulation of COUP-TFII gene expression during the stage of branching lung morphogenesis may cause pulmonary hypoplasia by repressing RSP.

Despite prenatal diagnosis and improved postnatal treatment strategies, the mortality and morbidity in patients with congenital diaphragmatic hernia (CDH) remains high [1,2]. Pulmonary hypoplasia, characterized by immaturity and small lung size, is considered to be one of the principle contributors to the high morbidity and mortality in infants with CDH [1,3].

Nitrofen-induced CDH has been used as an experimental model and has provided important insights into the pathogenesis of this developmental anomaly [4-7]. Recent studies have suggested that the retinoid signaling pathway

Presented at the 55th Annual Congress of the British Association of Paediatric Surgeons, Salamanca, Spain, July 2-5, 2008.

* Corresponding author. Children's Research Centre, Our Lady's Hospital for Sick Children, Dublin 12, Ireland. Tel.: +353 1 4096420; fax: +353 1 4550201.
E-mail address: prem.puri@ucd.ie (P. Puri).

0022-3468/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.jpedsurg.2008.10.079

(RSP) is inhibited in the nitrofen-induced hypoplastic lung [8-11]. However, the exact mechanism by which nitrofen acts in the RSP remains unclear.

COUP-TFII, chicken ovalbumin upstream promoter-transcription factor II, is a member of the steroid/thyroid hormone receptor superfamily and encodes a transfactor regulated by the RSP [12,13]. COUP-TFII is expressed in the developing lung, the foregut mesenchyme, the developing posthepatic mesenchymal plate, and the septum transversum, all components that are important for the formation of the diaphragm [14]. Homozygous tissue-specific ablation of COUP-TFII in mice has been shown to cause left-sided posterolateral CDH similar to Bochdalek-type CDH seen most commonly in humans [14]. COUP-TFII gene is located on chromosome 15q26. Recently Kantarci and Donahoe [15] reported that deletion of COUP-TFII gene is strongly associated with CDH. Although COUP-TFII is critical for the normal development of the diaphragm and lung, the exact role of COUP-TFII in lung development is unknown. It has been shown that COUP-TFII has 2 main roles in the RSP: (i) repressing of the RSP by directly sequestering retinoid X receptors (RXRs), thereby preventing heterodimerization to retinoid acid receptors (RARs) and inhibiting gene transcription, and (ii) interacting physically with FOG2, which, in turn, modulates the transcriptional activity of GATA4, GATA5, and GATA6 [16,17].

We designed this study to investigate the hypothesis that the pulmonary COUP-TFII expression is up-regulated during lung morphogenesis in the nitrofen CDH model, causing pulmonary hypoplasia by repressing RSP.

1. Materials and methods

1.1. Animals and drugs

Adult Sprague-Dawley rats were mated, and the presence of spermatozoids in the vaginal smear was verified and was considered as gestational day 0. Pregnant female rats were then randomly divided into 2 groups. Animals in the experimental group received intragastrically 100 mg of Nitrofen (Wako Chemicals, Osaka, Japan) dissolved in 1 mL of olive oil on day 9 of gestation, whereas those in the control group received only vehicle. Fetuses were delivered by caesarean section on day 15 (D15), D18, and D21 of gestation. Fetuses exposed to nitrofen were defined as the nitrofen group (n = 9 at each time-point). The control group (n = 9 at each time-point) consisted of animals that only received olive oil. The Department of Health and Children approved all the animal experiments (ref. B100/3530) under the Cruelty to Animals Act, 1876, as amended by European Communities Regulations 2002.

1.2. mRNA isolation and real-time reverse transcription–polymerase chain reaction

The peripheral region of left lungs dissected from the thoracic cavity were immediately suspended in RNA*later* solution (Ambion, UK) and stored at −20°C. The total RNA of each lung was extracted using TRIZOL reagent (Life Technologies, Railey, UK) according to recommended protocol. Reverse transcription (RT) was performed using RETROscript (Ambion, USA) according to manufacturer's instruction. Polymerase chain reaction (PCR) was performed using LightCycler 480 SYBR Green I Master (Roche Diagnostics, Germany) according to the manufacturer's protocol. The specific primer sets used in this study are listed (Table 1). After RT at 50°C for 30 minutes, 45 cycles of amplification were carried out (denaturation at 95°C for 15 seconds, annealing at 58°C for 30 seconds, and extension at 72°C for 30 seconds). Relative levels of gene expression was measured by LightCycler 480 (Roche Diagnostics, Germany) according to the manufacturer's instruction. Serial dilution of one sample RNA was prepared to create a standard curve for the relative quantification of mRNA in the samples. Experiments were carried out in triplicate for each data point. The relative changes in levels of specific genes were expressed as a percent of the control values that were set equal to 100%, after the normalization by the level of β-actin expression in each sample.

1.3. Statistical analysis

All numerical data are presented as means ± standard deviation. Differences between 2 groups at each gestational day were tested by using an unpaired Student or Welch t test when the data had normal distribution or Mann-Whitney U test when the data deviated from normal distribution. Statistical significance was accepted at P values less than .05.

2. Results

The relative mRNA expression levels of COUP-TFII at D15 was significantly increased in the nitrofen group

Table 1 Quantitative real-time RT-PCR primers

Gene	Sequence	Product size (bp)
β-actin		108
Forward	TTG GAT GCC TGT GGT CTG TC	
Reverse	TAG AGC CAC CAA TCC ACA CA	
COUP-TFII		124
Forward	TCA GAT GCC TGT GGT CTG TC	
Reverse	AAA GCT TTC CGA ACC GTG T	