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# Disturbance of retinol transportation causes nitrofen-induced hypoplastic lung

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**Index words:**
Nitrofen;
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**Abstract**

**Purpose:** Retinoids play a key role in lung development. Recent studies suggest that retinoid signalling pathway may be disrupted in the nitrofen model of congenital diaphragmatic hernia (CDH), but the exact mechanism is not clearly understood. We hypothesized that nitrofen interferes with cellular uptake of retinol during lung morphogenesis and therefore designed this study to examine total retinol levels in lung, liver, and serum, and the gene expression of main components of the retinoid pathway in the nitrofen model of CDH.

**Methods:** Pregnant rats were exposed to vehicle or 100 mg of nitrofen on day 9 of gestation. Term fetuses were divided in control and nitrofen with CDH and without CDH groups. Retinol levels in serum, lungs, and liver were measured using high-performance liquid chromatography. Reverse transcriptase–polymerase chain reaction was performed to evaluate the relative amount of cellular retinol-biding protein I, retinal dehydrogenase 1a2 and 1a3 (Aldh1a2 and Aldh1a3), retinoic acid receptors $\alpha$ and $\beta$ (RAR$\alpha$, RAR$\beta$), and retinoid X receptor $\alpha$ (RXR$\alpha$) expression in the lung.

**Results:** Total retinol levels in the lungs were significantly lower in both nitrofen with CDH ($1.78 \pm 0.37$ $\mu$g/g) and nitrofen without CDH ($1.61 \pm 0.24$ $\mu$g/g) groups compared with controls ($2.43 \pm 0.31$ $\mu$g/g) ($P < .001$), whereas serum retinol levels were significantly higher in nitrofen with and without CDH groups ($0.77 \pm 0.13$ and $0.75 \pm 0.11$ $\mu$g/g, respectively) compared with controls ($0.58 \pm 0.12$ $\mu$g/g) ($P < .001$). There was no significant difference in liver retinol levels between the 3 groups. Relative expression of cellular retinol-biding protein I, Aldh1a3, RAR$\alpha$, RAR$\beta$, and RXR$\alpha$ were significantly up-regulated in the lungs of the nitrofen with CDH group ($0.70 \pm 0.15$, $3.94 \pm 0.91$, $2.15 \pm 0.47$, $3.49 \pm 1.00$, $1.88 \pm 0.42$, respectively) and the nitrofen without CDH group ($0.61 \pm 0.14$, $3.72 \pm 0.31$, $1.66 \pm 0.20$, $3.28 \pm 1.02$, $1.38 \pm 0.24$, respectively) compared with controls ($0.43 \pm 0.11$, $2.71 \pm 0.47$, $0.79 \pm 0.42$, $1.85 \pm 0.69$, $0.57 \pm 0.22$, respectively) ($P < .05$).

**Conclusion:** Our data clearly show that lung retinol storage is decreased in the nitrofen model of CDH. The associated increase in gene expressions of most downstream components of the retinoid signalling

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pathway may be a feedback reaction to the deficiency of lung retinol. These results suggest that nitrofen acts by interfering with the cellular uptake of retinol during lung morphogenesis resulting in pulmonary hypoplasia in this model.


Despite significant advances in neonatal resuscitation and intensive care, newborn infants with congenital diaphragmatic hernia (CDH) continue to have high mortality and morbidity. Pulmonary hypoplasia in CDH, characterized by immaturity and small size, produces respiratory failure that is considered to be the principal contributor to the high mortality [1]. Much of the current understanding of pathogenesis of CDH originates from experimental studies. A teratogenic model of CDH in rodents has been widely used. Maternal exposure to nitrofen (2,4-dichlorophenyl-*p*-nitropheniyl ether) in both mouse and rat models during a specific time in gestation results in a high rate of CDH and associated pulmonary hypoplasia to their embryos, which is strikingly similar to the human condition [2]. Although the nitrofen model of CDH has been widely used, the exact pathogenesis of pulmonary hypoplasia, particularly in the nitrofen-induced animals without CDH, is not clearly understood.

Retinoids, vitamin A and its derivatives, are essential for growth, development, and tissue differentiation [3]. The major sources of vitamin A in the human diet are the provitamin A carotinoids in fruits and vegetables and retinyl esters found in food of animal origin. After absorption through the gut, retinyl esters are transported in chylomicrons to the liver for storage, where they are metabolized into retinol. To meet tissue needs for retinoid, the liver secretes retinol bound to retinol-biding protein (RBP) into the plasma, where it forms the main transporting complex with transthyretin. Circulating retinol is delivered to target tissues via a specific membrane receptor [4-6]. Within cells, retinol bound to cellular retinol-biding protein I (CRBP-I) is oxidized to retinal (Fig. 1). Retinal is oxidized to active metabolite, retinoic acid (RA), by retinal dehydrogenase. Currently, there are 3 known retinal dehydrogenases, RALDH1, RALDH2, and RALDH3, renamed as Aldh1a1, Aldh1a2, and Aldh1a3. Aldh1a2 and Aldh1a3 are essential for RA synthesis, whereas Aldh1a1 is involved in the catabolism of excess retinol [7]. Retinoic acid exerts its biologic effects through binding nuclear receptors, the retinoic acid receptor (RAR) and retinoid X receptor (RXR), of which there are 3 types of each; $\alpha$, $\beta$, and $\gamma$. Most of the body's reserve of vitamin A is stored in the liver as retinyl esters; other sites of major vitamin A include the eye and the lung [6].

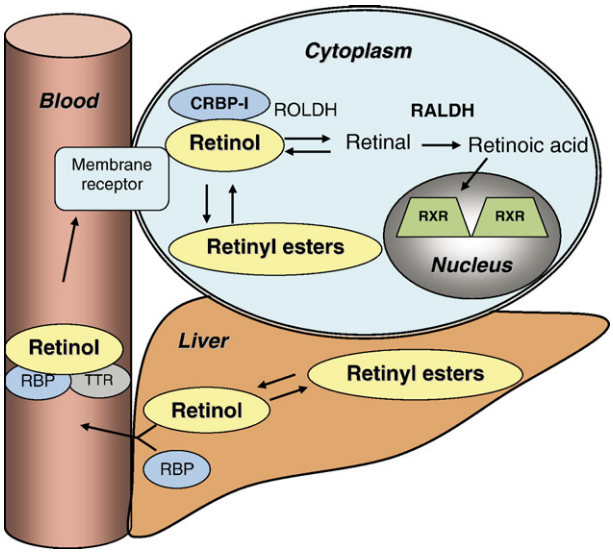


**Fig. 1** Schematic overview of the retinoid pathway. TTR indicates transthyretin; ROLDH, retinol dehydrogenase.

Recent studies suggest that retinoid signalling pathway may be involved in pathogenesis of CDH and associated pulmonary hypoplasia. The first evidence linking retinoids with CDH was published in 1941. The authors found that there was 25% incidence of CDH in the litters born to dams with vitamin A–deficient diets [8]. A small clinical study revealed that the infants with CDH had lower plasma retinol levels compared with healthy infants [9]. A study using genetically engineered mice has shown a pronounced suppression of retinoic acid response element by nitrofen [10]. Various abnormalities reported in RAR$\alpha$/RAR$\beta$ double knockout mice include diaphragmatic hernia [11]. An in vitro assay has demonstrated that nitrofen inhibits RALDH2, the enzyme catalyzing the final step in retinoic acid production [12]. Antenatal administration of vitamin A reduced the incidence of CDH and also restored lung maturation in the nitrofen rat model [13-15]. Furthermore, we have recently demonstrated that retinoic acid improves nitrofen-induced hypoplastic lung growth using fetal lung rat explants [16]. The above studies suggest that the retinoid pathway may be involved in the pathogenesis of CDH and associated pulmonary hypoplasia.

We hypothesized that nitrofen interferes with cellular uptake of retinol during lung morphogenesis and therefore designed this study to examine total retinol levels in lung, liver, and serum, and the gene expression of main components of the retinoid pathway in the nitrofen model of CDH.

## 1. Materials and methods

### 1.1. Animal model

Adults Sprague-Dawley rats were mated overnight. Twelve hours later 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 100 mg