

A molecular treatment paradigm for congenital diaphragmatic hernia

Edwin Soto Jr.^{1*}

Congenital diaphragmatic hernia (CDH) is a life-threatening disease in which the contents of the abdomen encroach upon the heart and lungs of the developing fetus. Despite many advances in managing the condition, it still persists as a major cause of neonatal death in newborns with severe respiratory failure. This review provides a brief context of CDH including its impact, clinical manifestations, and current management & limitations. A possible molecular treatment paradigm is also discussed based on studies linking retinoid signaling to CDH development. This evidence will outline the involvement of retinoid signaling pathways in lung & diaphragm development, the disruption of retinoid signaling resulting in CDH development, and a potential therapeutic modality by the manipulation of the retinoid signaling pathway.

INTRODUCTION

Congenital diaphragmatic hernia (CDH) is a condition that is classically described as a "failed closure of the pleuroperitoneal canals between the eighth and tenth week of gestation" (Montedonico *et al.*, 2008). This creates an opening between the suprapertitoneal cavity and the inferior thoracic wall early in development. The intestine protrudes into the chest cavity and encroaches upon the lungs and heart; leading to pulmonary hypoplasia and pulmonary hypertension (Keckler & Schropp, 2010). The vast majority of these cases (90% to 95%) occur posterolaterally (Bochdalek-type; Torfs *et al.*, 1992). In addition to this, CDH occurs once out of every 2,500 births and is responsible for 8% of all major congenital anomalies (Torfs *et al.*, 1992). Even with many advances in intensive care management, such as extracorporeal membrane oxygenation (ECMO) or high-frequency oscillatory ventilation (HFOV), the survival rates have been reported to be as low as 40% (Stege *et al.*, 2003). These treatment modalities have also had a limited impact on the morbidities associated with survivors of CDH (Ivascu & Hirschl, 2004; Doyle & Lally, 2004). This review briefly presents the current therapeutic strategies adopted for CDH management and discusses its limitations given the current progress. Additionally, a feasible alternative to the current treatment modality will be proposed based on several studies linking retinoid signaling to CDH development.

Clinical Manifestations

An infant with CDH typically presents at birth with an increased work of breathing, tachypnea, tachycardia, and cyanosis (Keckler & Schropp 2010). Inspection of the chest and abdomen may reveal asymmetrical chest rise and an unusually "caved-in" belly (scaphoid abdomen; Black, 2010). Borborygmus may sometimes be appreciated on the affected side of the chest (Black, 2010).

¹Florida State College, 501 W. State St., Jacksonville, FL 32202, USA.

*To whom correspondence should be addressed. Email: edwinsotojr@gmail.com.

Blood gas analysis tends to indicate hypoxia, hypercapnia, and acidosis (Keckler & Schropp, 2010); while a chest x-ray may show "cyst-like" opacities on the affected side caused by herniated air-filled loops of intestine that protrude into the chest cavity (Ingram, 2010).

Current treatment paradigm

The management of CDH can be summarized in four stages. The first stage is to stabilize the infant immediately following birth. This includes any rescue efforts. If an infant is suspected of having CDH during a resuscitation attempt, endotracheal intubation is done immediately, in lieu of bag-mask ventilation (Keckler & Schropp, 2010; Black, 2010). This is to avoid gastric distention, which can prove deleterious to breathing and circulation. In addition to this, a nasogastric tube is placed in order to allow the bowels to decompress and the lungs to better expand (Black, 2010). The second stage involves critical care management. The infant is admitted to the neonatal intensive care unit (NICU) where stabilization can be maintained and improvement monitored. Central to this strategy is the management of the patient's ventilation. Currently, mechanical ventilation (MV), high-frequency oscillatory ventilation (HFOV), and extracorporeal membrane oxygenation (ECMO) are all viable options for CDH. Regardless of which option is used, the goal is to maintain adequate ventilation & oxygenation, in an attempt to prevent hypercapnia, hypoxemia, and pulmonary hypertension while avoiding barotrauma. Common practice is to avoid high airway pressures and prevent pulmonary vasospasm by keeping the blood alkalotic (PaCO₂ between 25-30mmHg). After the patient is given time to adapt to postnatal life, the third stage of CDH management can be employed: surgical repair. The herniation is corrected with a transabdominal approach that reconfigures the intestinal viscera to their proper orientation and closes the opening with suturing or a prosthetic patch. Unfortunately, surgical repair does not reverse pulmonary hypertension or hypoplasia, because they are the consequence of a molecular, not a structural, aberration resulting from CDH development. In order to monitor these pathologies, a fourth stage of management is employed: immediate and long-term

postoperative care. The lungs and mediastinum are given time to resolve to their proper position and common complications, such as increased pulmonary vascular resistance (PVR), are focused on in their management. Even after the infant has recovered enough for the parents to take home, long-term follow-up is needed to manage chronic lung disease which arises as a result of CDH. (Keckler & Schropp, 2010)

A molecular treatment paradigm for CDH

The primary limitation for how CDH is currently managed is that the disease has progressed considerably before an intervention is applied. This is not a new observation, since surgeons have attempted to correct CDH earlier in fetal development by

The metabolic goal for consuming dietary vitamin A is to obtain a source of retinyl esters. These are absorbed in the gut and transported to the liver for storage (Figure 1-A). When the tissues require retinyl esters, it is converted into a transportable form (retinol) and attached to two transport proteins: RBP (retinol-binding protein) and TTR (transthyretin) (Figures 1-B and 1-C). This complex of proteins is then able to travel in the plasma where it can bind to the cell surface receptors of the target cell for internalization (Figure 1-D). Once inside the cell, retinol can take on two forms, retinyl ester or retinal. The enzyme ROLDH (retinol dehydrogenase) facilitates the conversion of retinol into the more metabolically useful form, retinal (Figure 1-E). Retinal is then converted into the final product of metabolic activity, retinoic acid, by another enzyme:

performing *in utero* repair (Harrison, 1990). However, even these worthy attempts have not improved survival rates significantly (Keckler and Schropp 2010). As will become apparent, the issue with CDH is that it is a disease that occurs on a molecular level which in turn produces a structural defect. Surgical repair may correct the structural anomaly, but it does not correct the molecular aberration. This is why survivability is unlikely to improve with surgical intervention and why it does little to reverse pulmonary hypertension & hypoplasia. This limitation, however, could potentially be overcome by the application of a treatment paradigm that corrects the etiologic cause of the disease by intervening at the biomolecular level where it initially develops.

RALDH (retinal dehydrogenase; see Figure 1-F). Retinoic acid can then bind to two ligand-activated transcription factors within the nucleus: RAR (retinoic acid receptor) and RXR (retinoid X receptor; Figure 1-H). When bound to these two proteins, it activates the complex and is responsible for an up-regulation of proteins involved in growth, development, and differentiation (see Figure 1-I). The entire pathway is kept in check by the protein Cyp26 which breaks down retinoic acid into metabolites (see Figure 1-G) (Montedonico *et al.*, 2008).

Several studies have implicated retinoid-signaling in lung and diaphragm development. This is especially important since pulmonary hypoplasia has been shown to occur concomitantly with CDH rather than as a result of direct compression by the encroaching intestines (Keijzer *et al.*, 2000; Babiuk & Greer, 2002). Among some of the more interesting findings related to lung development is that retinoid signaling is involved in every stage of lung development (Montedonico *et al.*, 2008). Lung development occurs in five phases: embryonic, pseudoglandular, canalicular, saccular, and alveolar (Schnapf & Kirley, 2010). During the embryonic phase, the trachea arises out of an endodermal bud from the foregut which grows caudally to form the precursors of the trachea and two mainstem bronchi (Schnapf & Kirley, 2010). Several studies have demonstrated expression of RALDH2 and retinoid receptors (RAR β and RXR α /RXR β) during this early period in pulmonary development (Malpel *et al.*, 2000; Dole *et al.*, 1990; Dole *et al.*, 1994). Moreover, retinoic acid has been shown to have a central role in FGF10 (fibroblast growth factor 10) expression in the foregut region where the initial budding occurs during early lung morphogenesis (Desai *et al.*, 2004). The pseudoglandular phase is characterized by continuous branching of the conducting airways (Schnapf & Kirley, 2010). Beyond this stage, no further branching is observed and continued growth occurs only by elongation and widening of the airways (DiFiore & Wilson, 1994). Retinoic acid is thought to inhibit distal branching and promote differentiation of the proximal buds during this stage (Maden, 2004). The canalicular phase is named after the appearance of vascular beds that begin to grow during this period while the saccular phase is distinguished by the thinning of the airspace walls and the production of surfactant (Schnapf & Kirley, 2010). The alveolar phase begins following birth and terminates between 18 and 24 months (Schnapf & Kirley, 2010).

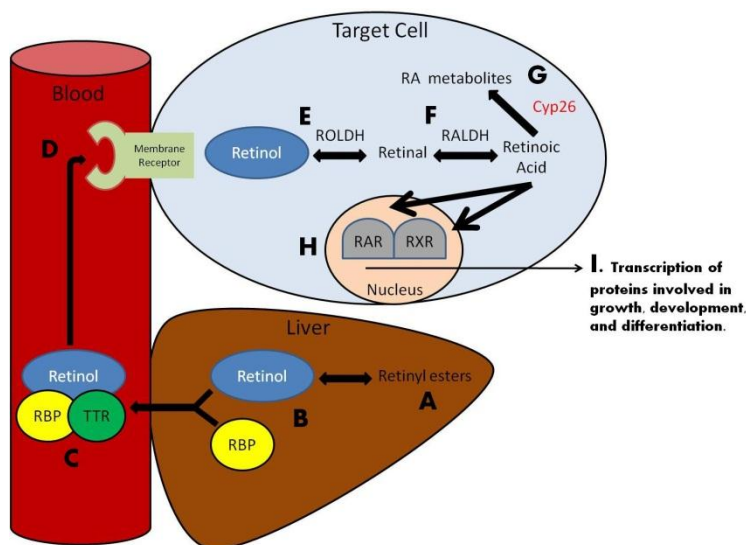


Figure 1. A. Physiologic retinoid signaling entails the conversion of retinyl esters obtained from the gut into retinol. B and C. Retinol then forms a transport complex with retinol-binding protein (RBP) and transthyretin (TTR). D. The complex travels through the bloodstream where it is internalized by the target cell via a membrane receptor. E and F. Once inside the cell, retinol is converted into retinoic acid by two enzymes: retinol dehydrogenase (ROLDH) and retinal dehydrogenase (RALDH). G. Cyp26 is a physiologic inhibitor of retinoic acid. H. The transcription factors RAR (retinoic acid receptor) and RXR (retinoid X receptor) are activated by binding to retinoic acid respectively. I. This results in the expression of proteins that are important to growth, development, and differentiation. Figure adapted from Montedonico et al. 2008.

During this time both the number of functional alveolar units and the tensile strength of the lung increase (Schnapf & Kirley, 2010). Interestingly, the transcription factors RAR and RXR have been shown to aid in the differentiation of epithelial cells in the canalicular, saccular, and alveolar phases (reviewed in Maden 2004).

With respect to diaphragm development, RALDH2, RBP, and RAR β are all strongly expressed in the pleuroperitoneal folds of developing rat diaphragms (Mey *et al.*, 2003). Two early studies showed that up to 70% of pups born to rats bred on a vitamin A deficiency developed CDH (Andersen, 1941; Wilson *et al.*, 1953). These rates of herniation were reduced when vitamin A was reintroduced (Andersen, 1941; Wilson *et al.*, 1953).

What is more significant is that a study done by Noble and colleagues has demonstrated a pathogenic mechanism for the classic teratogenic model of CDH (nitrofen-induction) involving retinoid-signaling (Noble *et al.*, 2007; Greer *et al.*, 2000). They observed depressed retinoic acid levels as a result of induction with nitrofen (teratogen); a finding that was consistent with the work of Mey *et al.*, which concluded that nitrofen disrupted RALDH2 activity (Mey *et al.*, 2003). Another study found that genetically engineered mice having the lacZ-reporter gene linked to a retinoic response element (RAR-E), showed marked suppression of expression when exposed to nitrofen as well (Chen *et al.*, 2003). This suppression was then rescued with supplemental retinoic acid. Moreover, Thebaud *et al.* demonstrated that dosing vitamin A with nitrofen reduced the incidence of CDH in rats from 84% to 40% along with a decrease in pulmonary hypoplasia (Thebaud *et al.*, 1999). Taken together, these findings suggest that the retinoid-signaling pathway maybe implicated in CDH development.

Treating CDH on a molecular level

It is currently feasible to treat CDH on a molecular level using several techniques and technologies already in use for other applications. The first step, from a practical perspective, would be to adequately screen pregnant mothers. This would give clinicians the advantage of early detection which is critical to an effective intervention for CDH. Mothers should be screened for any factors that may lead to fetal vitamin A deficiency such as malnutrition or an inadequate diet. Perhaps the best way to accomplish this is to check the maternal and fetal blood for optimum retinol-RBP-TTR complex levels as it is the final functional product that is transported to the fetus. One study has already pointed to the possible merits of screening (Major *et al.*, 1998). Major and colleagues found that there was a 50% reduction in retinol and RBP in the cord blood of infants found to have CDH when compared to those that did not. In addition to this, they also found that the mothers of these infants had high levels of retinol/RBP; suggesting that the development of human CDH may arise out of a defect in the placental barrier. Therefore, a simplistic intervention such as a direct infusion into the fetal circulation may prove to be beneficial to correct the disease in humans. Currently, *in-utero* endoscopic (fetoscopic) surgery offers a promising route for delivery of therapeutic

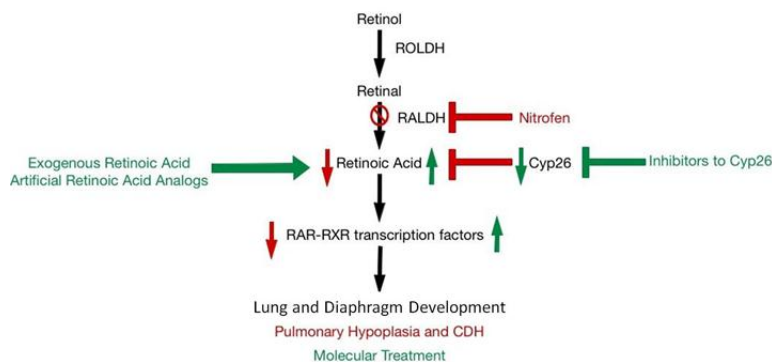


Figure 2. Molecular treatment of CDH. Black represents normal retinoid-signaling (Montedonico *et al.* 2008). Retinol is converted into retinal by the enzyme ROLDH (retinol dehydrogenase). Retinal is then converted into retinoic acid by the enzyme RALDH (retinal dehydrogenase). Retinoic acid then activates the transcription factors RAR (retinoic acid receptor) and RXR (retinoid X receptor) by binding to them. This leads to the up-regulation of proteins that are important to normal lung and diaphragm development. Cyp26 is a physiologic inhibitor of retinoic acid. Red represents how pulmonary hypoplasia and CDH occur (Montedonico *et al.* 2008; Noble *et al.* 2007). Nitrofen inhibits the enzymatic activity of RALDH which blocks the conversion of retinal into retinoic acid. The inhibition of retinoic acid is further strengthened by Cyp26. With no retinoic acid available to bind and activate RAR & RXR, there is a down-regulation of the proteins that are important to normal lung and diaphragm development. This results in pulmonary hypoplasia and CDH. Green represents how Molecular Treatment could stop or reverse pulmonary hypoplasia and CDH. Exogenous retinoic acid, artificial retinoic acid analogs that bind & activate RAR-RXR, or inhibitors to Cyp26 rescues the down-regulation of retinoic acid caused by nitrofen induction. This would result in the activation of RAR-RXR which would increase the expression of proteins involved in normal lung and diaphragm development.

agents to the fetus (Deprest *et al.*, 2010). Biochemically, a multi-pronged approach could be taken to ensure there is an up-regulation of the proteins that are produced by the retinoid-signaling pathway. This could be accomplished by introducing a combination of exogenous retinoic acid, artificial retinoic acid analogs that constitutively bind and activate RAR/RXR, or inhibitors to proteins that down-regulate the buildup of retinoic acid (Figure 2). Artificial retinoic acid analogs have already found a niche as agents used to treat diseases like psoriasis, acne, and certain cancers (Thacher *et al.*, 2000). It could prove beneficial to study the effects of these agents in the context of CDH development.

CONCLUSION

As discussed before, the current therapeutic interventions for CDH are limited in their ability to improve the developmental derangement as they apply a structural correction to an aberration which occurs on a molecular level. Molecular treatment attempts to resolve this shortcoming by identifying the pathway involved and then implementing a strategy that will correct this, allowing complete physiological development. The evidence presented not only implicates retinoid signaling in normal lung & diaphragm development but also demonstrates that its disruption may lead to CDH development. Given these results, it is possible to propose a therapeutic strategy that rescues normal expression of proteins involved in lung and diaphragm development using current techniques and technologies. Future

study should be directed at finding the human mechanism for retinoid signaling in CDH development and whether the

molecular interventions explored in this review would prove beneficial.

REFERENCES

- Andersen, D. (1941) Incidence of congenital diaphragmatic hernia in the young of rats bred on a diet deficient in Vitamin A. *American Journal of Disease of Children* 62, 888-889.
- Babiuk, R. and J. Greer (2002) Diaphragm defects occur in a CDH hernia model independently of myogenesis and lung formation. *American Journal of Physiology- Lung Cellular and Molecular Physiology* 283, 1310-1314.
- Black, C. (2010) Examination and assessment of the neonatal patient. In B. Walsh, M. Czervinske, and R. DiBlasi (Eds). *Perinatal and Pediatric Respiratory Care* 3rd ed, 47-60.
- Black, C. (2010) Neonatal assessment and resuscitation. In B. Walsh, M. Czervinske, and R. DiBlasi (Eds). *Perinatal and Pediatric Respiratory Care* 3rd ed, 35-46.
- Chen, M. et al. (2003) The activation of the retinoic acid response element is inhibited in an animal model of congenital diaphragmatic hernia. *Biology of the Neonate* 83, 157-161.
- Deprest, J. et al. (2010) Fetal surgery is a clinical reality. *Seminars in Fetal & Neonatal Medicine* 15, 58-67.
- Desai, T. et al. (2004) Retinoic acid selectively regulates FGF10 expression and maintains cell identity in the prospective lung field of the developing foregut. *Developmental Biology* 273, 402-415.
- DiFiore, J. and J. Wilson (1994) Lung development. *Seminars in Pediatric Surgery* 3, 221-232.
- Dolle, P. et al. (1994) Developmental expression of murine retinoid X receptor (RXR) genes. *Mechanisms of Development* 45, 91-104.
- Major, D. et al. (1998) Retinol status of newborn infants with congenital diaphragmatic hernia. *Pediatric Surgery International* 13, 547-549.
- Malpel, S. et al. (2000) Regulation of retinoic acid signaling during lung morphogenesis. *Development* 127, 3057-3067.
- Mey, J. (2003) Retinal dehydrogenase-2 is inhibited by compounds that induce congenital diaphragmatic hernias in rodents. *American Journal of Pathology* 162, 673-679.
- Montedonico, S. et al. (2008) Congenital diaphragmatic hernia and retinoids: searching for an etiology. *Pediatric Surgery International* 24, 755-761.
- Noble, B. et al. (2007) Mechanism of action of the congenital diaphragmatic hernia-inducing teratogen nitrofen. *American Journal of Physiology Lung Cellular and Molecular Physiology* 293, 1079-1087.
- Schnapf, B. and S. Kirley (2010) Fetal lung development. In B. Walsh, M. Czervinske, and R. DiBlasi (Eds). *Perinatal and Pediatric Respiratory Care* 3rd ed, 2-12.
- Stege, G. et al. (2003) Nihilism in the 1990s: the true mortality of congenital diaphragmatic hernia. *Pediatrics* 112, 532-535.
- Dolle, P. et al. (1990) Retinoic acid receptors and cellular retinoid binding proteins. I. A systematic study of their differential pattern of transcription during mouse organogenesis. *Development* 110, 1133-1151
- Doyle, N. and K. Lally (2004) The CDH study group and advances in the critical care of the patient with congenital diaphragmatic hernia. *Seminars in Perinatology* 28, 174-184.
- Greer, J. et al. (2000) Recent advances in understanding the pathogenesis of nitrofen-induced congenital diaphragmatic hernia. *Pediatric Pulmonology* 29, 394-399.
- Harrison, M. et al. (1990) Correction of congenital diaphragmatic hernia in utero, V. Initial clinical experience. *Journal of Pediatric Surgery* 25, 47-55.
- Ingram, J. (2010) Radiographic assessment. *Perinatal and Pediatric Respiratory Care* 3rd edition, 91-103.
- Ivascu, F. and R. Hirschl (2004) New approaches to managing congenital diaphragmatic hernia. *Seminars in Perinatology* 28, 185-198.
- Keckler, S. and K. Schropp (2010) Surgical disorders in childhood that affect respiratory care. In B. Walsh, M. Czervinske, and R. DiBlasi (Eds). *Perinatal and Pediatric Respiratory Care* 3rd ed, 482-497.
- Keijzer, R. et al. (2000) Dual-hit hypothesis explains pulmonary hypoplasia in the nitrofen model of congenital diaphragmatic hernia. *American Journal of Pathology* 156, 1299-1306.
- Maden, M. et al. (2004) Retinoids in lung development and regeneration. *Current Topics in Developmental Biology* 61, 153-189.
- Thacher, S. et al. (2000) Therapeutic applications for ligands of retinoid receptors. *Current Pharmaceutical Design* 6, 25-58.
- Thebaud, B. et al. (1999) Vitamin A decreases the incidence and severity of nitrofen-induced congenital diaphragmatic hernia in rats. *American Journal of Physiology* 277, 423-429.
- Torfs, C. et al. (1992) A population-based study of congenital diaphragmatic hernia. *Teratology* 46, 555-556
- Wilson, J. et al. (1953) An analysis of the syndrome of malformations induced by maternal vitamin A deficiency. Effects of restoration of vitamin A at various times during gestation. *American Journal of Anatomy* 92, 189-217.