Antenatal Medical Therapies to Improve Lung Development in Congenital Diaphragmatic Hernia

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Abstract

Congenital diaphragmatic hernia (CDH) is a birth defect characterized by failed closure of the diaphragm, allowing abdominal viscera to herniate into the thoracic cavity and subsequently impair pulmonary and vascular development. Despite improving standardized postnatal management, there remains a population of severe CDH for whom postnatal care falls short. In these severe cases, antenatal surgical intervention (fetoscopic endoluminal tracheal occlusion [FETO]) may improve survival; however, FETO increases the risk of preterm delivery, is not widely offered, and still fails in half of cases. Antenatal medical therapies that stimulate antenatal pulmonary development are therefore interesting alternatives. By presenting the animal research underpinning novel antenatal medical therapies for CDH, and considering the applications of these therapies to clinical practice, this review will explore the future of antenatal CDH management with a focus on the phosphodiesterase-5 inhibitor sildenafil.

Keywords

- congenital diaphragmatic hernia
- antenatal therapy
- fetal
- sildenafil
- pulmonary hypertension of the newborn
- pulmonary hypoplasia

Congenital diaphragmatic hernia (CDH) is a birth defect characterized by failed closure of the diaphragm, allowing abdominal viscera to herniate into the thoracic cavity and subsequently impair pulmonary and vascular development.[1] Normal pulmonary development is predominantly driven by mechanical stretch,[2,3] fetal breathing movements,[4,5] and complex interactions between blood vessels and airways.[6] In CDH, pulmonary development is impaired, causing pulmonary hypoplasia and clinically resulting in respiratory insufficiency and pulmonary hypertension in the newborn,[7]

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which are major determinants of morbidity and mortality in CDH infants.[8]

Despite in utero referral, centralization of care and standardization of postnatal management,[9] there remains a population of severe CDH infants with extremely poor survival chances.[8,10] Overall, 20 to 30% of the 1 in 3,000 live-born neonates with isolated CDH still die, and in severe cases, survival is less than 15%.[11] In these cases, antenatal interventions may improve outcomes by increasing lung growth and possibly improving pulmonary structural development.[12] Until now, most strategies are based on fetal surgical interventions, and the current experimental approach is by fetoscopic endoluminal tracheal occlusion (FETO).[12] Though there is an apparent increase in survival rates, the success of fetal surgery is limited by technical feasibility, the major complication of preterm birth, and the requirement for patient access to experienced fetal surgical centres.[12] Apart from that, in the best case scenario up to 40% of fetuses will eventually not survive because of limited pulmonary response.[13] Because of these limitations, much research effort has been directed toward investigating alternative antenatal medical therapies. In 2015, Eastwood et al published a systematic overview of antenatal medical therapies under investigation in animal models of CDH, predominantly in the nitrofen rat model.[14] Several recent promising findings, particularly in relation to phosphodiesterase-5 inhibitors, warrant an update of this review that will take a more descriptive approach.

By presenting the animal research underpinning novel antenatal medical therapies for CDH, and considering the applications of these therapies to clinical practice, we will explore the future of antenatal CDH management.

Pathophysiology of Congenital Diaphragmatic Hernia

The pathogenesis of CDH remains unclear; however, the leading hypothesis is that abnormal development of the diaphragm's amuscular mesenchymal component results in an incomplete migration platform for muscle precursors.[15] The defect predominantly occurs on the left side (80–90%), the majority of which are located posterolaterally (70%).[1] An incomplete diaphragm allows herniation of abdominal contents into the thoracic cavity from the embryonic phase onward, from then disturbing further lung development.

The process by which CDH impairs pulmonary development is not entirely understood. The classical hypothesis is that herniating abdominal viscera occupies space in the thoracic cavity, placing external pressure on the developing lungs and interfering with fetal breathing movements.[16] Alternatively, Keijzer et al proposed the "dual-hit" hypothesis: early in development a genetic or environmental insult prevents closure of the diaphragm and also impairs bilateral pulmonary branching morphogenesis. Following this initial insult, the herniating abdominal viscera further contributes to pulmonary hypoplasia.[17]

In addition to reduced lung size and airway complexity,[7] increased muscularization and decreased cross-sectional area of distal pulmonary vessels lead to increased pulmonary vascular resistance.[18] Normally at birth, air entry drives lung liquid out of the airways and into the surrounding tissue, which triggers a decrease in pulmonary vascular resistance and subsequent increase in pulmonary blood flow and oxygenation.[19,20] In contrast, pulmonary vascular resistance remains high at birth in most neonates with CDH, often leading to clinically significant pulmonary hypertension.[21] This adds significant postnatal morbidity, as in most cases, infants with pulmonary hypertension need mechanical ventilation to treat pulmonary hypertension-induced hypoxemia, which may contribute to further lung injury. Neonates with CDH and severe pulmonary hypertension have mortality rates ranging from 56.1 to 100%.[8,10,22] Therefore, preventing pulmonary hypertension by identifying and treating these patients in utero may be key to improving their survival.

Limitations of Antenatal Surgical Therapies for Congenital Diaphragmatic Hernia

Tracheal ligation was first described as a method of enhancing lung growth by preventing egress of lung liquid, in 1965.[23] After intensive preclinical experimentation, tracheal occlusion was first achieved clinically in an open procedure that required hysterotomy and fetal neck dissection.[24] This open procedure was associated with severe neurological morbidity and a survival rate of 33%; therefore, efforts were made to investigate less invasive approaches.[24] Initially, this included a maternal laparotomy and multiple trocar insertions, but gradually this evolved to a percutaneous single port procedure that was optimized by the FETO consortium.[25–27]

In 2009, the FETO consortium published its experience of using FETO for isolated CDH in more than 200 cases. Indeed, survival rates were higher than was anticipated based on historical controls. However, despite the use of keyhole surgery (3.3 mm single trocar), preterm premature rupture of membranes (47.1%) and subsequently preterm birth (median gestational age at delivery: 35.3 weeks) are still important complications.[12] Nevertheless, these promising results lead to two large multicenter randomized trials evaluating the efficacy of FETO (www.totaltrial.eu).[28,29] Within these trials, the balloon is inserted at 27 to 30 weeks of gestation in severe cases of CDH and 30 to 32 weeks of gestation in moderate cases.[30]

The current clinical strategy includes reversal of tracheal occlusion because experimental work has shown that if the balloon is not removed, then surfactant deficiency results from transdifferentiation of surfactant-producing type II alveolar epithelial cells into nonsurfactant-producing type I cells.[31–33] Balloon removal also allows the patient to return to the institution where she would normally deliver and potentially have a vaginal delivery. Elective balloon removal is typically scheduled for 34 weeks of gestation either by ultrasound-guided percutaneous puncture or by fetoscopy.[34] A recent series described that in experienced centers in 96.8% of elective procedures, the balloon can be successfully

removed.[35] In 28 to 43% of cases, however, obstetrical complications and, in particular, threatened preterm labor prompted an emergent removal of the balloon.[12,35] In this emergency setting, if release cannot be performed by percutaneous puncture or fetoscopy, extraction may be required on placental circulation. Balloon extraction was initially performed as a formal ex utero intrapartum procedure, yet now is safely done during a modified cesarean section.[12,35,36] As a last resort, the balloon can be removed postnatally with a specially designed tracheoscope or percutaneous puncture.[34] Difficulties in balloon removal played a major contribution to neonatal death in 10 of the 210 cases performed by the FETO consortium;[12] therefore, it is recommended that a 24/7 team of experienced clinicians are on-call to deal with emergency balloon removal.[30] This limits the use of FETO to high-volume tertiary referral centers.

Finally, the success of FETO appears dependent on preexisting lung size, potentially because smaller lungs have less lung liquid secreting epithelium.[12] Furthermore, as lung liquid secretion ceases when the fetal lung's intraluminal pressure reaches ~6 mm Hg,[37] if the pressure required to expand the hypoplastic lung exceeds 6 mm Hg, then FETO will not increase lung expansion. These limitations indicate that further antenatal medical therapies are required to address the remaining morbidity and mortality of severe CDH, preferentially medical in nature to reduce invasiveness and make prenatal therapy more accessible.

Antenatal Medical Therapies for Congenital Diaphragmatic Hernia

This review will predominantly focus on the antenatal medical therapies that show the most promise in correcting pulmonary hypoplasia and pulmonary hypertension in CDH —corticosteroids, retinoids, and phosphodiesterase-5 inhibitors. Several other medical therapies for CDH have been investigated and are extensively reviewed by Eastwood et al.[14]

Recently, there have also been promising results using cell-based therapies to improve pulmonary development in CDH.[38–40] In contrast to medical therapies, cell-based therapies may not only prevent further lung injury but also repair established disease.[41] Regenerative medicine could contribute to the management of CDH on various levels, as recently reviewed by De Coppi and Deprest,[42] but it is beyond the scope of this article.

Corticosteroids

The only antenatal medical therapy for CDH studied in a human randomized controlled trial is the corticosteroid betamethasone, which was administered by intramuscular injection to mothers at 34 weeks of gestation (two doses of 12.5 mg 24 hours apart, then 12.5 mg weekly for 2 weeks).[43] An interim analysis after 32 completed cases (17 steroid, 15 placebo) showed no differences in perinatal mortality, mechanical ventilation time, or days of hospital admission. Based on these preliminary results, the data safety monitoring committee decided to end the study prematurely, as it was determined that to demonstrate any significant difference more than 1,700 fetuses with a prenatally diagnosed CDH would need to be enrolled. The experience of this trial highlights that as a rare condition in which only a subgroup of the most severely afflicted will obtain benefit from antenatal therapy, randomized controlled trials of CDH are only feasible if conducted in a co-ordinated world-wide multicenter approach.

Mechanism of Action

Research efforts initiated by Liggins and Howie[44] and summarized by Ballard and Ballard[45] demonstrated that the timing of fetal lung maturation correlates with a physiological increase in circulating corticosteroids, and that administering antenatal exogenous corticosteroids accelerates lung development. Antenatal corticosteroids improve neonatal lung function by acting at a transcriptional level. The mechanism of action was initially described as accelerating the maturation of alveolar structure, cell differentiation, surfactant production, and lung liquid clearance mechanisms;[46] however, findings from glucocorticoid receptor knockout mice indicate that glucocorticoids predominantly improve lung maturation by restraining hyperproliferation of the interalveolar septum and surrounding mesenchyme.[47,48] The benefits of antenatal corticosteroids to lung development have led to their extensive use to reduce disease associated with developmental immaturity in the setting of threatened preterm delivery, recently summarized by Kemp et al.[49] Observations by George et al that infants with CDH had structurally immature lungs suggested that antenatal corticosteroids may also improve lung development in the setting of CDH.[50]

Pharmacokinetics and Bioavailability

The efficacy of antenatal corticosteroids for CDH was first established in successful rat,[51–54] rabbit,[55–57] and sheep[58,59] models (►**Table 1**). These animal studies predominantly investigated betamethasone and dexamethasone, fluorinated synthetic corticosteroids that cross the placenta from maternal to fetal circulation, have minimal mineralocorticoid activity and are weak immunosuppressants.[49] In the setting of threatened preterm delivery, betamethasone is administered intramuscularly in two 12 mg doses, 24 hours apart.[60] This generates a maximum fetal plasma concentration of ~20 ng/mL, 1 to 2 hours after treatment, with a half-life of 12 hours in the fetal circulation.[45] The maximum maternal plasma concentration of ~100 ng/mL is reached 1 hour after treatment, with a half-life of 6 hours.

Safety

Antenatal corticosteroids are extensively used to improve fetal lung maturation for women with threatened preterm delivery, thereby reducing neonatal mortality and respiratory morbidity. A recent Cochrane review including 30 randomized controlled trials found that antenatal corticosteroid treatment does not increase the risk of neurodevelopmental delay, chorioamnionitis, endometritis, or maternal

Table 1 Antenatal steroids in experimental animal models of congenital diaphragmatic hernia

Domain	Impact of antenatal steroids	
Gross lung size (LBWR)	No significant effect[51–53,55,56,63]	
Airway morphometry	↓ Septal thickness[51,53,58,59]	
	↑ Airspace volume[51,53,58,59]	
	↑ Radial alveolar count[58]	
Vascular Morphometry	↓ Medial wall thickness[53,56,57]	
	↑ Distal vessel number[56]	
Biochemical changes	Unclear effect on surfactant protein expression	No change[51]
		↑[64]
	Unclear effect on eNOS expression	No change[51]
		↑[53]
	Unclear effect on growth factors and proliferation markers	↑ bFGF, PDGF, TGF-β1 expression[54]
		↓ K_i-67 and PCNA mRNA[63]
	Unclear effect on glycogen levels	No change[55]
		↓[59]
	Increases VEGF and VEGF receptor expression	↑[52,67]
		↓[53]
Physiological changes	↑ Postductal PaO_2[59]	
	↑ Dynamic compliance[59]	

Abbreviations: bFGF, basic fibroblast growth factor; eNOS, endogenous nitric oxide synthase; LBWR, lung-to-body weight ratio; mRNA, messenger ribonucleic acid; PCNA, proliferating cell nuclear antigen; PDGF, platelet-derived growth factor; TGF-β1, transforming growth factor β1; VEGF, vascular endothelial growth factor.

death, and reduces the risk of perinatal and neonatal death.[61] It found no definitive evidence suggesting differences between a single course and weekly repeated corticosteroids; however, the Maternal-Fetal Medicine Units Network raises concerns regarding a nonstatistically significant ($p = 0.12$) association between multiple doses of antenatal corticosteroids and cerebral palsy.[62]

Effects on Airways in Diaphragmatic Hernia

Antenatal corticosteroids appear to accelerate lung maturation, but there are concerns that this may be at the expense of lung growth. Accelerated lung maturation is indicated by decreased septal thickness, increased airspace volume, and increased radial alveolar count.[51,53,58,59] On the contrary, lung:body weight ratio appears unaffected by antenatal corticosteroids, and messenger ribonucleic acid (mRNA) expression of proliferation markers K_i-67 and proliferating cell nuclear antigen is reduced.[63] Promisingly, corticosteroids appear to primarily inhibit mesenchymal cell proliferation, which may provide benefit by enhancing perisaccular septation.[47]

The impact of antenatal steroids on surfactant production in CDH is unclear; Davey et al[64] demonstrated that antenatal betamethasone partially restored surfactant protein mRNA expression in sheep, whereas Suen et al[51] found no effect in the rat. It is also unclear whether antenatal corticosteroids enhance[56,65] or reduce[63] the beneficial effects of tracheal occlusion. In current clinical programs, corticosteroids are used due to threatened preterm delivery at around the time of balloon removal (usually at 34 weeks), hence it is often forgotten that they are part of the current clinical prenatal strategy and should be included in experimental models of tracheal occlusion.

Effects on Pulmonary Vasculature in Diaphragmatic Hernia

Antenatal corticosteroids improve the vasodilation response of pulmonary blood vessels by increasing expression of endothelial nitric oxide synthase and K^+ channels;[53,66] however, their effect on vascular proliferation is not yet fully understood. At a biomolecular level, antenatal corticosteroids initially appeared to increase vascular endothelial growth factor (VEGF) receptor expression;[52,67] however, Gonçalves et al found that in ventilated lungs, dexamethasone was associated with decreased expression of VEGF and its vasodilatory receptor Flk-1.[53] Morphologically, antenatal corticosteroids increase distal vessel number and decrease distal vessel muscularization.[53,56,57]

Translation from Animal Models to Clinical Practice

At this time, there does not appear to be additional benefit in providing antenatal steroids to CDH fetuses, other than the well-established benefits of antenatal steroids for infants expected to deliver prior to 34 weeks.[9]

Retinoids

Retinoids are a family of molecules derived from vitamin A, an essential micronutrient required for organogenesis, reproduction, immune competence, cellular differentiation, and vision. The retinoid signaling pathway appears important in normal diaphragmatic and pulmonary development, and it is hypothesized that disruptions in this pathway may contribute to the pathogenesis of CDH.[68]

Pharmacokinetics, Bioavailability, and Mechanism of Action

Vitamin A is obtained from the diet as retinyl esters in meat or β-carotene in vegetables, which are then metabolized to retinol in the liver. After being secreted by the liver and transported by retinol binding protein in the blood, retinol enters cells and is eventually converted to the active metabolite, retinoic acid. Retinoic acid enters the nucleus and regulates gene transcription.

In the lungs, retinoid signaling is an important component of lung budding and branching early in development, and later influences septation and alveolarization.[69]

The retinoid signaling pathway also appears to play an important role in complete closure of the diaphragm. Diaphragmatic defects are present in the offspring of vitamin A–deficient rats[70] and retinoic acid receptor double knockout mice,[71] and infants with CDH have low levels of plasma retinol.[72,73]

In the rat model of CDH, the herbicide nitrofen is used to induce diaphragmatic hernia. Noble et al[74] suggest that nitrofen acts by inhibiting the enzymes that convert retinol to retinoic acid, whereas Nakazawa et al[75] suggest that nitrofen interferes with the cellular uptake of retinol. Importantly, both hypotheses indicate that in nitrofen-induced diaphragmatic hernia, administering vitamin A or retinol will not necessarily increase intracellular levels of the active metabolite, retinoic acid. Considering this, retinoic acid appears to be a more appealing therapeutic agent than vitamin A itself.

Furthermore, while neonatal plasma retinol levels are low in CDH, maternal plasma retinol levels are either equivalent to controls or elevated.[72,73] This suggests impaired placental transfer of retinoids in the setting of CDH; therefore, vitamin A administered to the mother may never reach the fetus. In contrast, retinoic acid appears to cross the placenta in the nitrofen rat model of CDH, as it exerts a therapeutic effect.[76,77]

Safety

The use of exogenous retinoids to prevent pulmonary hypoplasia in CDH requires caution, as retinoid toxicity is associated with teratogenic effects such as spontaneous abortion and cranial neural crest defects.[78] Rothman et al estimate that among mothers who ingest more than 10,000 IU of vitamin A supplements per day, 1 in 57 infants are born with a birth defect attributable to vitamin A.[78]

The dangers of retinoid teratogenicity are demonstrated by cases in which isotretinoin, used to treat severe acne, has inadvertently been taken during pregnancy. Lammer et al[79] determined that of 154 pregnancies exposed to isotretinoin, 95 were aborted electively, 12 spontaneously aborted, and 21 were born with malformations.

Isotretinoin is the 13-cis isomer of retinoic acid and exerts species-dependent teratogenic effects. An extensive body of work undertaken by Nau[80] can be summarized as follows. 13-cis-retinoic acid has a long half-life (16 hours) and extensive access to cell nuclei, however, limited placental transfer. Some, but not all, of the teratogenicity of 13-cis-retinoic acid can be attributed to its continuous isomerization to all transretinoic acid, which has a short half-life (1 hour) but extensive placental transfer and high affinity for retinoic acid receptors. In humans, exogenous 13-cis-retinoic acid is more teratogenic than exogenous all trans-retinoic acid because continuous isomerization during its long half-life results in higher total fetal exposure to toxic levels of all transretinoic acid.

The teratogenic potency of 13-cis-retinoic acid is more in humans than in rats, likely because the rodent metabolic pathway more efficiently eliminates 13-cis-retinoic acid and placental transfer is almost nonexistent in rodents.[80] In rodents, all transretinoic acid is more teratogenic than 13-cis-retinoic acid.

Studies in the nitrofen rat have administered all transretinoic acid via intraperitoneal injection at a dose of 5 mg/kg/d, however, have not reported rates of craniofacial, thymic, or cardiac malformations.[76,77] Further investigations into potential teratogenicity in the rat, rabbit, and sheep models of CDH are required before antenatal retinoids should be considered for use in humans.

Effect on the Lungs in Diaphragmatic Hernia

The effect of retinoids on lung development varies depending on the time of administration. When administered late in lung development (E16–20 in the nitrofen rat; term = 21 days), antenatal retinoids improved structural maturation without affecting lung growth; the size and number of airspaces were increased (increasing surface area available for gas exchange), distal artery muscularization was decreased, and there was increased expression of VEGF and its receptors, but the lung:body weight ratio was not improved.[76,77] In contrast, when administered early in lung development (E 9– 14 days in the nitrofen rat), antenatal retinoids improved both lung growth and maturation; the lung:body weight ratio was increased and surfactant protein expression was increased.[81,82]

In the nitrofen rat model, a chemical "first-hit" impairs lung growth early in development. Later, once the diaphragmatic defect has allowed herniation of abdominal viscera, a "second-hit" further impairs pulmonary development. Only the "first-hit" is directly related to nitrofen-related impairment of the retinoid signaling pathway, which explains why only early administration of retinoids improves lung growth. Findings from surgical models of CDH are consistent with this hypothesis. In the rabbit model, lung growth was unaffected by antenatal retinoids, and in the sheep model, antenatal retinoids were associated with a decreased lung:body weight ratio.[83,84]

While late antenatal retinoids did not improve lung growth, they did appear to improve lung maturation in nitrofen rats. This likely reflects the importance of retinoid signaling in

septation and alveolarization; thinner interalveolar septa and improved alveolarization were also seen in the sheep model of CDH.[84] These structural changes were associated with improved lung function after delivery of the lambs. On the contrary, septation and alveolarization were unaffected by antenatal retinoids in the rabbit model of CDH.[83]

Translation from Animal Models to Clinical Practice

To improve both lung growth and maturation in the nitrofen rat, antenatal retinoids needed to be administered during the pseudoglandular stage of lung development.[85] In humans, the pseudoglandular stage is complete by ~18 weeks.[85] Given that CDH is predominantly diagnosed at 20 weeks or later in gestation,[86] it may not be possible to administer vitamin A at a stage early enough to improve lung:body weight ratio. However, it is sometimes possible to diagnose CDH in first-trimester screening programs, yet severity assessment has not been validated that early in pregnancy, and the prognosis of these infants is unclear.[87]

Ultimately, vitamin A remains a promising antenatal therapy to improve pulmonary development in CDH that is worthy of further investigation in animal experiments; however, concerns regarding teratogenicity must be addressed and further advances in early antenatal diagnosis are required before it can be considered in a clinical trial.

Phosphodiesterase-5 Inhibitors

The previously discussed antenatal medical therapies display limited benefit, may be teratogenic (retinoic acid), or have failed to demonstrate benefit in humans despite promising animal trials (corticosteroids). In contrast, the phosphodiesterase-5 inhibitor sildenafil has been extensively used in animal and human studies for other maternal–fetal conditions, and has demonstrated promising structural and biochemical pulmonary vasculature changes in small animal models of CDH.[88–95]

Sildenafil's extensive use in erectile dysfunction[96] means that it is now available off-label in a much cheaper generic form. Along with consistent oral bioavailability,[97] this allows sildenafil to be used in low-resource settings—in stark contrast to the highly specialized care required for other antenatal CDH interventions such as FETO.[30,36]

Sildenafil is already used clinically in the postnatal management of persistent pulmonary hypertension of the newborn in CDH neonates,[9] and has been proven to be safe when given to pregnant women for oligohydramnios, or within trials for growth restriction, however, applying its effects antenatally to improve pulmonary development would be an exciting step forward.

Mechanism of Action

Phosphodiesterases are a group of enzymes that play an important role in regulating cardiac smooth muscle tone and vascular smooth muscle contraction.[98] Sildenafil is a relatively selective inhibitor of one of these enzymes, phosphodiesterase-5. By degrading cyclic guanosine monophosphate (cGMP), phosphodiesterase-5 interferes with the nitric oxide–mediated vasodilation process and hence results in vasoconstriction. Sildenafil-induced phosphodiesterase-5 inhibition leads to an increase in the half-life of cGMP, hence vasodilation and increased blood flow (►**Fig. 1**).

Phosphodiesterase-5 is the most active phosphodiesterase in the pulmonary vasculature, hence sildenafil induces pulmonary vasodilation.[99] Pulmonary vasodilation reduces pulmonary vascular resistance, which increases pulmonary blood flow. This mechanism of action allows sildenafil to effectively treat pulmonary hypertension in adults[100] and children,[101] and there is also early evidence in neonatal populations.[102] These patients are particularly sensitive to the effects of sildenafil because phosphodiesterase-5 is upregulated in pulmonary hypertension.[99]

Similarly, phosphodiesterase-5 is upregulated in CDH lungs at birth;[103] therefore, CDH infants are often nonresponsive to inhaled nitric oxide.[104] Sildenafil's acute vasodilatory effect may appear less useful in utero, when gas exchange is not required and pulmonary blood flow is minimal. Yet, in addition to a steady increase in pulmonary blood flow throughout gestation, there are dynamic increases in pulmonary blood flow during accentuated fetal breathing movements.[105] Sildenafil-mediated vasodilation may therefore enhance these episodes by allowing more blood to flow through the lungs during fetal breathing movements, suggesting a potential role in utero for sildenafil's acute vasodilatory effect.

However, in utero the long-term effects of sildenafil are likely more important. While its effects on pulmonary vascular resistance and pulmonary blood flow are transient, antenatal sildenafil mediates a sustained change in vascular reactivity to birth-related stimuli (such as hyperoxemia) that persists to delivery.[106] Sildenafil also exerts an antiproliferative effect on pulmonary artery smooth muscle cells, so may reduce the increased distal artery muscularization characteristic of CDH.[107] Decreased muscularization may be due to upregulation of antiproliferative bone morphogenetic protein.[108] Furthermore, phosphodiesterase-5 suppresses VEGF expression, so by inhibiting phosphodiesterase-5, sildenafil may also improve VEGF-driven angiogenesis and therefore restore the reduced number of distal vessels seen in CDH.[109] VEGF-driven angiogenesis also creates mature alveolocapillary units[110] that will appropriately match ventilation and perfusion during neonatal life, reflecting the complex interactions between airway and vascular pulmonary development discussed earlier.[111]

Sildenafil may exert these long-term effects directly, or they may be secondary to increased cGMP levels.[107] Interestingly, increased cGMP inhibits phosphodiesterase-3 (a cAMP-specific phosphodiesterase) and hence also leads to increased cAMP levels.[112] cAMP is known to have antiproliferative effects on smooth muscle, which may explain sildenafil's effect on distal arterial musculature.[112]

Pharmacokinetics and Bioavailability

The impact of antenatal sildenafil on fetuses with CDH has been predominantly studied in the nitrofen rat model[88,89,91–95] and also one rabbit study has been published so far (►**Table 2**).[90] An alternative phosphodiesterase-5 inhibitor, tadalafil, has recently been investigated in the sheep model.[113]

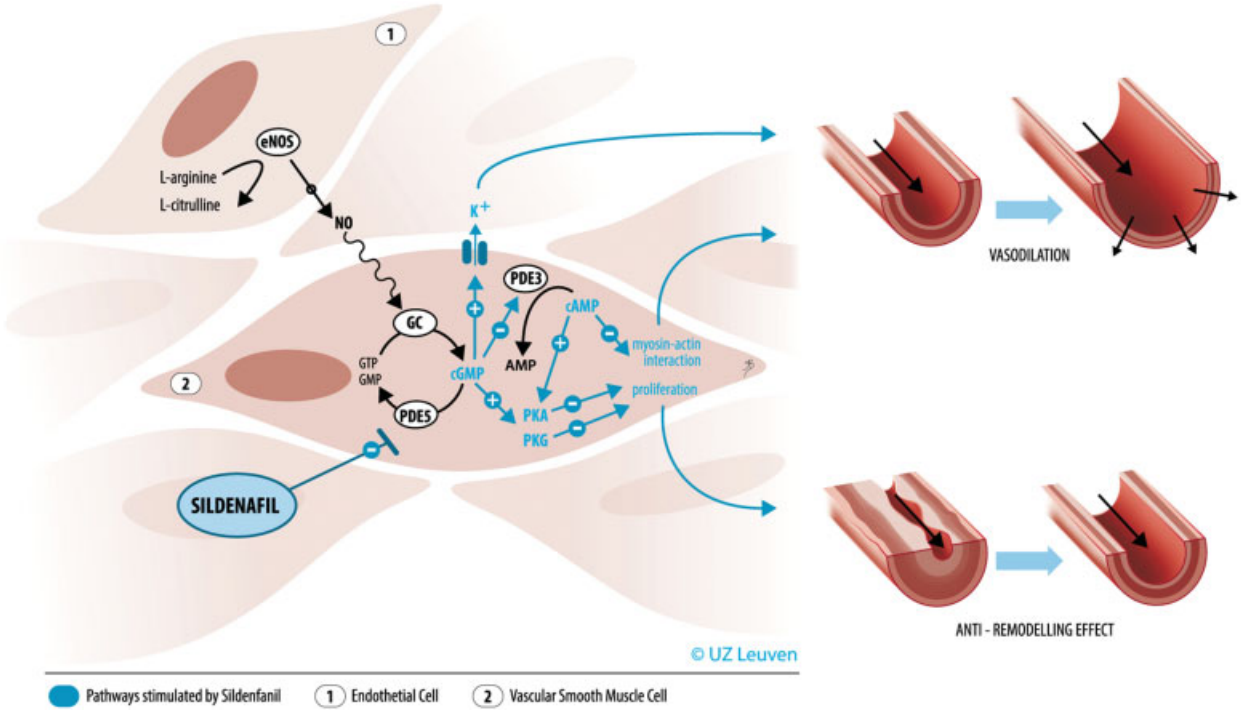

Fig. 1 Schematic representation of the mechanism of action of sildenafil on the pulmonary vasculature. AMP, adenosine monophosphate; ATP, adenosine triphosphate; cAMP, cyclic AMP; cGMP, cyclic GMP; eNOS, endothelial nitric oxide synthase; GC, guanylate cyclase; GMP, guanosine triphosphate; GTP, guanosine triphosphate; NO, nitric oxide; PDE, phosphodiesterase; PKA, cAMP-dependent protein kinase; PKG, cGMP-dependent protein kinase. (Reproduced with permission from UZ Leuven, Leuven, Belgium. Drawing by Myrthe Boymans.)

Tadalafil is a more potent and selective phosphodiesterase-5 inhibitor; therefore, a lower dose is required to achieve the same effect relative to sildenafil.[113] Furthermore, its longer half-life means that serum levels would be more consistent with daily oral dosing. Unfortunately, human neonates are unable to properly metabolize tadalafil due to an immature glucuronidation pathway; therefore, it is unlikely to be used clinically as an antenatal therapy.[114] Nevertheless, when sildenafil treatment was compared with tadalafil in neonates with persistent pulmonary hypertension, both were shown to decrease mean pulmonary artery pressure with no significant difference between the two treatments.[115]

The pharmacokinetics of sildenafil differ between species, mostly due to rate of metabolism.[116] To obtain plasma levels within the therapeutic range (47–500 ng/mL[90]), rabbits require 10 mg/kg/d and rodents require 100 mg/kg/d (►**Table 2**).[90–95]

In both species, there is excellent bioavailability following both oral and subcutaneous administration, hence either

Table 2 Antenatal sildenafil in experimental models of congenital diaphragmatic hernia

Author, y	Dose	Route	Timing
Rat models (gestational term: ED 22)			
Luong et al, 2011[92]	100 mg/kg/d Sildenafil	Subcutaneous injection	ED 11.5–20.5
Kattan et al, 2014[95]	45 mg/kg BD Sildenafil	Oral	ED 14–22
Lemus-Varela et al, 2014[91]	100 mg/kg/d Sildenafil	Oral	ED 16–20
Yamamoto et al, 2014[94]	100 mg/kg/d Sildenafil	Subcutaneous injection	ED 11.5–20.5
Makanga et al, 2015[93]	100 mg/kg/d Sildenafil	Oral	ED 11–21
Burgos et al, 2016[88]	100 mg/kg/d Sildenafil	Subcutaneous injection	ED 11.5–20.5
Mous et al, 2016[89]	100 mg/kg/d Sildenafil	Oral	ED 17.5–20.5
Rabbit model (gestational term: GA 31)			
Russo et al, 2016[90]	10 mg/kg/d Sildenafil	Subcutaneous injection	GA 24–30
Sheep model (gestational term: GA 145)			
Shue et al, 2014[113]	2 mg/kg/d Tadalafil	Oral	GA 75–135

Abbreviations: ED, embryonic day; GA, days of gestational age.

method is appropriate.[116] In the majority of rodent models, sildenafil was administered from the pseudoglandular stage onward;[88,91–95] however, in the most recent study, sildenafil treatment only began during the more clinically relevant canalicular stage (the stage during which human CDH is usually detected at 18–20 weeks ultrasound).[89] In rabbits, it was administered from the canalicular stage onward.[90]

The first important finding from these studies is that maternal sildenafil successfully crosses the placenta in all current animal models. Interestingly, in sheep, fetal tadalafil concentrations remain at a steady state despite fluctuating maternal levels (►**Fig. 2**).[113] This steady state may reflect low fetal metabolism of tadalafil in sheep fetuses, as in human neonates.[114] These steady fetal concentrations are not seen when sildenafil is used in the rat[92] and rabbit[90] models. Sildenafil clearance in human neonates increases threefold (to adult levels) during the first week of postnatal life;[117] therefore, well-designed fetal pharmacokinetic studies are required to ensure that sildenafil does not accumulate in the fetal circulation when administered antenatally.

It is evident that transplacental sildenafil and tadalafil both exert a biochemical action on the fetal lungs, with an increase in pulmonary cGMP concentration following maternal administration in both the rat and sheep model.[92,113]

Safety

Human safety data specific to fetuses with CDH are not available for sildenafil, and there have been no adverse effects reported in animal models of CDH.[92]

In the neonatal population, using sildenafil to treat pulmonary hypertension was not associated with higher rates of any adverse effects in three small randomized controlled trials.[118–120] On the contrary, a randomized controlled trial investigating sildenafil in the pediatric population found that high-dose sildenafil is associated with greater mortality than low-dose sildenafil.[121] This association led to the U.S. Food and Drug Administration recommendation against the use of sildenafil in the pediatric population.[122] However, several expert groups have refuted this recommendation due to limitations of the trial, including inconsistent plasma sildenafil concentrations, no survival data for the placebo group, and a large number of confounding variables.[123,124] Furthermore, the European Medicines Agency approved sildenafil use in pediatric pulmonary hypertension (while warning against high doses) based on the same evidence.[125]

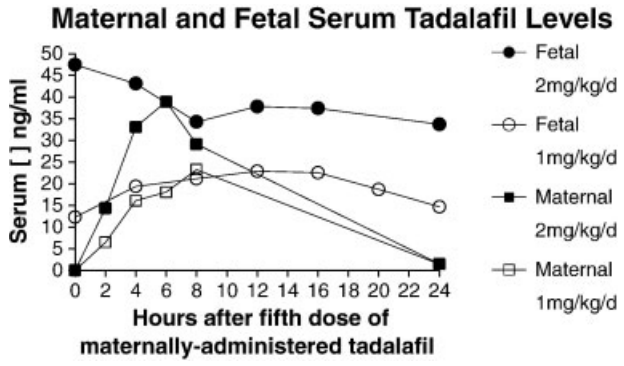

Fig. 2 Maternal and fetal serum tadalafil levels in the sheep model. Tadalafil was given maternally for 5 days, prior to recording data. (Reproduced with permission from Shue et al.)[113]

Antenatally, sildenafil has been investigated in randomized controlled trials to determine its effect upon pregnancy duration in women with preeclampsia,[126] and on amniotic fluid volume in pregnancies complicated by idiopathic oligohydramnios.[127] In both trials, no differences in adverse effects between sildenafil and placebo were observed, in either mother or fetus.

Antenatal sildenafil is also under investigation to increase placental blood flow in intrauterine growth restriction (IUGR) in the international STRIDER trial.[128] A recently published (2016) meta-analysis was unable to draw conclusions about the effect of antenatal sildenafil in IUGR, due to the lack of studies with placebo comparison,[129] but the ongoing randomized controlled trial has not reported any serious adverse effects to date.[128] Animal trials have raised controversy over whether sildenafil displays a beneficial[130] or detrimental[131] effect on the uteroplacental circulation. Concerns regarding adverse fetal effects have also been raised in an ongoing study in a sheep model of IUGR.[132] It is important that a safety profile is established in animal models of diaphragmatic hernia before sildenafil is considered for clinical trials in CDH.

Effect on the Lungs in Diaphragmatic Hernia

Sildenafil appears to be associated with several biomolecular changes, such as supporting a provasodilatory expression profile (►**Table 3**).[92,93,113] Sildenafil also restores the vasodilatory response to stimuli such as nitric oxide and oxygen that is impaired in CDH.[92,94] Furthermore, increases in bone morphogenic protein signaling (antiproliferative) and Bax/Bcl-2 ratio (proapoptotic) may underlie a reduction in vessel muscularization.[93] There is also increased expression of VEGF in the lung parenchyma, which may drive the observed increase in distal vessel number.[90] Sildenafil has no effect on phosphodiesterase-5 RNA expression but decreases the distribution of phosphodiesterase-5 particularly in distal pulmonary vessels.[89]

Sildenafil improves pulmonary vascular development in experimental models of CDH by increasing distal vessel number[90,92,93,95] and decreasing distal vessel muscularization (►**Table 4**).[89–93] Sildenafil did not significantly increase total vascular volume;[89] however, this is less important than the ratio of distal to proximal vessels (as a larger proportion of distal vessels provides greater cross-sectional area, therefore, lower resistance).

A concerning finding is that when sildenafil was given to control animals (i.e., without CDH), it decreased the number of distal vessels[90,92] and decreased total vascular volume.[89] The authors hypothesized that sildenafil-mediated vasodilation was beneficial in lungs with increased pulmonary vascular resistance, but in normal lungs, hypoperfusion of the pulmonary vascular bed may impair vessel growth.[90] These findings could alternatively be explained by the fact that while cGMP (increased following sildenafil-induced

Table 3 Antenatal sildenafil mediates biomolecular and physiological changes

Author, y	Vasodilator expression	Vasoconstrictor expression	Vasoreactivity to vasodilatory stimuli
Luong et al, 2011[92]	↑ eNOS ↑ VEGF	NA	↑ Vasodilatory response to NO donor
Yamamoto et al, 2014[94]	NA	NA	↑ Vasodilatory response to maternal hyperoxia
Makanga et al, 2015[93]	↑ eNOS ↑ iNOS ↑ NO	↓ ET1 ↓ ETA ↓ PPET1	NA
Burgos et al, 2016[88]	NA	NA	NA
Russo et al, 2016[90]	NA	NA	NA
Shue et al, 2014[113]	↑ eNOS	NA	NA

Abbreviations: eNOS, endothelial NO synthase; ET1, endothelin-1; ETA, ET1 receptor A; iNOS, inducible NO synthase; N/A, not applicable; NO, nitric oxide; PBF, pulmonary blood flow; PPET1, ET1 precursor; VEGF, vascular endothelial growth factor.

phosphodiesterase-5 inhibition) is important for vasodilation and angiogenesis, sustained exposure at high levels can lead to lung endothelial cell death and apoptosis.[133] While sildenafil may attenuate the reduced cGMP levels characteristic of CDH,[92] its use in healthy fetuses may increase cGMP to toxic levels.

Sildenafil also appears to have some beneficial effects upon airway development (►**Table 5**). The effect of sildenafil on gross lung size is unclear, with the majority of the evidence showing no difference,[90,92,93,113] one rat model demonstrating a significant decrease[88] and two rat models demonstrating a significant increase in lung weight.[89,94] At a histological level, sildenafil appears to increase distal airway complexity.[89,90,93,94] On a functional level, rabbit lungs treated with sildenafil demonstrate improved static compliance and total lung capacity.[90]

Translation from Animal Models to Clinical Practice

Most investigations into antenatal sildenafil in CDH have been performed in the nitrofen rat model, which does not allow assessment of the physiological transition at birth. Ultrasound assessments suggest that sildenafil reduces pulsatility index, an indicator of pulmonary vascular resistance.[88,90] In the larger sheep model, it was possible to demonstrate that tadalafil improved pulmonary blood flow during neonatal ventilation.[113] Despite no significant difference observed in mean pulmonary artery pressure, tadalafil did appear to improve pulmonary artery pressure response

Table 4 Antenatal sildenafil mediates changes in vascular morphology

Author, y	Vessel number (distal vessel number or density)	Vessel muscularization (medial wall thickness)	Other
Rat models (sildenafil)			
Luong et al, 2011[92]	In CDH: ↑ In non-CDH: ↓	↓	↓ Right ventricular hypertrophy
Kattan et al, 2014[95]	↑	Proximal vessels: ↑ Distal vessels: –	NA
Lemus-Varela et al, 2014[91]	NA	↓	NA
Makanga et al, 2015[93]	↑	↓	↑ Vessel diameter
Burgos et al, 2016[88]	NA	No effect	NA
Mous et al, 2016[89]	NA	↓	↓ Cellular markers of muscularization
Rabbit model (sildenafil)			
Russo et al, 2016[90]	In CDH: ↑ In non-CDH: ↓	↓	NA
Sheep model (tadalafil)			
Shue et al, 2014[113]	NA	NA	No effect on right ventricular hypertrophy

Abbreviations: CDH, congenital diaphragmatic hernia; N/A, not applicable.

Table 5 Antenatal sildenafil mediates changes in airway histology and function

Author, y	Gross lung size	Septal thickness	Airway complexity	Functional
Rat models (sildenafil)				
Luong et al, 2011[92]	No effect	↓	NA	NA
Lemus-Varela et al, 2014[91]	NA	No effect	No effect	NA
Yamamoto et al, 2014[94]	↑ LBWR	NA	↑ Radial saccular count	NA
Makanga et al, 2015[93]	No effect	No effect	↑ Radial alveolar count	NA
Burgos et al, 2016[88]	↓ LBWR	↓	↑ Alveolar volume density No effect on radial alveolar count	↑ PO_2 No effect on tidal volume
Mous et al, 2016[89]	↑ LKWR	No effect	↑ Alveolar airspace diameter	NA
Rabbit model (sildenafil)				
Russo et al, 2016[90]	No effect	No effect	↑ Distal airway complexity	↑ Static compliance ↑ Total lung capacity
Sheep model (tadalafil)				
Shue et al, 2014[113]	No effect	NA	NA	NA

Abbreviations: LBWR, lung-to-body weight ratio; LKWR, lung-to-kidney weight ratio (used because body weight was significantly different between groups in this study); NA, not applicable; PO_2, partial pressure of oxygen (from a mixed arteriovenous sample).

to inhaled nitric oxide.[113] Further experiments should be conducted in the sheep model to verify these results, and assess their relevance to sildenafil. Meanwhile, we are preparing to move to clinical trials and have recently obtained orphan designation for antenatal sildenafil for CDH by the European Medicines Agency.

Future Directions

Although FETO is limited by complications associated with preterm delivery and technical difficulties, it does significantly improve lung growth and may be associated with a significant decrease in morbidity and mortality in CDH.[12,28] To overcome the limitations while retaining the benefits of FETO, antenatal medical therapies should be investigated not only as alternatives to tracheal occlusion but also for potential synergistic effects. Mixed results from animal experiments make it unclear whether betamethasone and vitamin A have synergistic effects with tracheal occlusion;[64,134–136] however, promising early results from a rabbit model of CDH indicate that there may be synergistic effects of antenatal sildenafil and tracheal occlusion on vascular and parenchymal lung development.[137] FETO improves lung growth and sildenafil appears to improve pulmonary vascular development, so further investigating synergistic effects between the two is an important avenue for future research.

Conclusion

Novel approaches to CDH management are urgently required to further reduce the significant mortality associated with severe CDH because postnatal management occurs too late to prevent lung hypoplasia and pulmonary hypertension. Antenatal surgical approaches are limited by high rates of preterm birth and high technical complexity limiting availability. Of the currently investigated antenatal medical therapies for CDH, the phosphodiesterase-5 inhibitor sildenafil appears the most promising candidate for a future clinical trial. Small animal studies have demonstrated that antenatal sildenafil attenuates vascular remodeling in utero;[90–95] however, further research is required to determine if this leads to a functional improvement during the neonatal cardiovascular transition. We are hopeful that one day a novel antenatal medical therapy such as sildenafil will offer a new treatment modality in the care of pregnancies with severe CDH.

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