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EMERGING ANTENATAL THERAPIES FOR CONGENITAL DIAPHRAGMATIC HERNIA-INDUCED PULMONARY HYPERTENSION IN PRECLINICAL MODELS

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Abstract

Congenital diaphragmatic hernia (CDH)-related deaths are the largest contributor of in-hospital neonatal deaths in children with congenital malformations. Morbidity and mortality in CDH are directly related to the development of pulmonary hypertension (PH). Current treatment consists of supportive measures. To date, no pharmacotherapy has been shown to effectively reverse the hallmark finding of pulmonary vascular remodeling that is associated with pulmonary hypertension in CDH (CDH-PH). As such, there is a great need for novel therapies to effectively manage CDH-PH. Our review aims to evaluate emerging therapies, and specifically focuses on those that are still under investigation and not approved for clinical use by the Food and Drug Administration. Therapies were categorized into antenatal pharmacotherapies or antenatal regenerative therapies and assessed on their method of administration, safety profile, effect on pulmonary vascular pathophysiology, and overall efficacy. In general, emerging antenatal pharmaceutical and regenerative treatments primarily aim to alleviate pulmonary vascular remodeling by restoring normal function and levels of key regulatory factors involved in pulmonary vascular development and/or in promoting angiogenesis. Overall, while these emerging

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therapies show great promise for the management of CDH-PH, most require further assessment of safety and efficacy in preclinical models before translation into the clinical setting.

INTRODUCTION

Congenital diaphragmatic hernia (CDH) is a highly morbid birth defect that occurs in approximately 1 in 3,000 live births (1). Despite advancements, the etiology remains unclear in over 50% of patients. CDH consists of a diaphragmatic defect, dextrocardia, lung hypoplasia, and often, pulmonary hypertension (PH) (figure 1A). In gestation the diaphragm fails to develop properly, allowing abdominal organs to herniate into the chest cavity (figure 1B) and impede normal lung development; furthermore, lung hypoplasia (figure 1D) and compromised pulmonary vascular development (figure 2) lead to PH, resulting in respiratory failure, right heart failure, and even death (2). As such, pulmonary hypertension in CDH (CDH-PH) is a major determinant of disease-related morbidity and mortality (2–4). CDH-related deaths account for the highest number of in-hospital neonatal deaths in infants with congenital malformations (5), with a striking difference in survival rates between CDH infants with and without PH (20% *vs.* 70%) (6). Management of the disease is expensive, with estimated costs ranging from $250–800 million every year (7, 8).

Current treatment consists of supportive measures such as gentle mechanical ventilation, vasodilators, supplemental oxygen, and extracorporeal life support, which aim to minimize cardiopulmonary symptoms. No available pharmacotherapy effectively reverses the vascular remodeling process that is responsible for the structural vascular alterations observed in CDH-PH (2). Not surprisingly, no available therapy significantly improves CDH morbidity and mortality related to PH (2, 9). Given the considerable impact this disease poses on our neonatal population, a novel therapy is clearly necessary. Our review aims to evaluate emerging therapies that are still under investigation and not approved for clinical use by the Food and Drug Administration.

PATHOPHYSIOLOGY

CDH-PH pathogenesis is still not fully understood. A two-hit hypothesis (10) proposes that an initial embryological insult disrupts lung development, followed by external compression of the ipsilateral lung from the herniated abdominal organs. This suggests that both intrinsic genetic and molecular defects, as well as extrinsic mechanical forces, are responsible for CDH-PH pathology and the clinical cardiopulmonary changes that occur after birth. CDH infants develop a reduced number of pulmonary vessels, and their remaining vessels are impaired by vascular remodeling processes that induce medial wall thickening and distal muscularization (figure 3). An imbalance in vasodilatory and vasoconstrictive mediators further impedes signaling between dysfunctional endothelial and pulmonary arterial smooth muscle cells, resulting in increased pulmonary vascular resistance and elevated pulmonary arterial pressures consistent with PH. Evidence demonstrates that disruptions to key regulatory pathways involved in normal vascular development are associated with CDH-PH pathogenesis.

ANTENATAL CDH-PH PHARMACOTHERAPIES

To date, all CDH-PH pharmacotherapies are delivered postnatally and only address pulmonary artery vasoconstriction as part of supportive management. Some theorize that interventions are administered too late, as vascular remodeling begins *in utero* (11, 12). An antenatal approach that addresses vascular changes early in development may mitigate disease before it progresses. Importantly, since lung development in humans continues through at least the second year of life, and per some authors through seven years of life, postnatal interventions are still necessary to achieve satisfactory outcomes. Furthermore, prenatal therapies present an ethical dilemma due to the possibility of unintended side effects for both the mother and the fetus, thus potentially harming two lives instead of just the targeted patient. For this reason, postnatal interventions must continue to be explored, and antenatal therapies appropriately evaluated for maternal risk. Here, we discuss only antenatal therapies (table 1), and evaluate their effects on molecular pathways.

Nitrofen-induced CDH rodents are the most commonly used animal model to investigate antenatal therapies, as they develop surprisingly similar pathology compared to CDH infants. Much like humans, a portion of the litter will have large diaphragmatic defects containing herniated abdominal organs and have associated bilateral lung hypoplasia (13). Histopathological analysis reveals arterial wall thickening, a hypoplastic vascular bed, and reduced airway branching consistent with human CDH pathophysiology (14) (figure 4).

Therapies targeting cGMP pathways

Nitric oxide (NO) is key for endothelial cell function and vascular regulation. It is produced by endothelial nitric oxide synthase (eNOS) in pulmonary endothelial cells and activates soluble guanylate cyclase to release cyclic guanosine monophosphate (cGMP) within the vascular smooth muscle cells. Activation of cGMP-dependent pathways increases vasodilation, anti-thrombotic activity, and cellular proliferation (2, 15). Endothelial-derived NO production increases in response to signaling from vascular endothelial growth factor (VEGF), an angiogenic factor that promotes endothelial cell migration and proliferation (15), and stimulates angiogenesis (16, 17). Studies show both increased (18) and decreased (19–22) VEGF expression in nitrofen-induced rat lungs. In contrast, phosphodiesterase enzymes (PDE) regulate NO pathway activity via cGMP inactivation.

Animal and human CDH studies report conflicting results regarding molecular changes within the NO pathway (15). Some nitrofen-induced CDH rat models show decreased pulmonary levels of NO (23) and eNOS (21, 23–25), while others show increased eNOS levels (26, 27). Similarly, human CDH studies demonstrate decreased (28) and increased (29) NOS levels in pulmonary vessels. In nitrofen-exposed fetal rat lungs, the pathway is further impaired by increased PDE expression and diminished reactivity to NO and cGMP stimuli (30). This dampened cellular response may be attributed to prolonged NO exposure, as may occur in the setting of increased eNOS or inducible nitric oxide synthase (iNOS) expression, because this has been shown to reduce both soluble guanylate cyclase activity and cGMP levels in pulmonary artery smooth muscle cells (31). These findings suggest that pathological downregulation of the NO-cGMP pathway contributes to CDH-PH.

Phosphodiesterase inhibitors

Sildenafil: Sildenafil is a phosphodiesterase type 5 (PDE5) inhibitor used for adult and pediatric PH (32–35), including CDH-PH, and particularly for NO-refractory disease (26, 36–38). PDE5 inhibitors improve pulmonary vasodilation, oxygenation (37), cardiac output, and reduce pulmonary vascular resistance in PH patients (22, 39). Antenatal administration was first explored in nitrofen-induced CDH rats. The treatment arm received subcutaneous sildenafil from embryonic day (E)11.5 until E20.5 (22). Fetuses were delivered at term. Results showed increased pulmonary angiogenesis, improved vasoreactivity, and decreased right ventricular hypertrophy following sildenafil administration. Histology confirmed no adverse visual effects or brain impairment. Comparable results showing attenuated vascular remodeling with reduced arterial muscularization (23, 26, 40, 41) and increased vessel density (23, 42) were reproduced in other nitrofen-induced rat models, and in a surgical diaphragmatic hernia rabbit model (43). Sildenafil-treated rats had increased levels of eNOS (22, 23), iNOS (23), and VEGF, with reductions in active PDE5 (22). Although VEGF levels were not upregulated in treated rabbits, enhanced vasorelaxation in response to VEGF stimulation was significantly increased (43). This suggests that sildenafil restores pulmonary vasculature and lung morphology via normalization of NO-cGMP-dependent pathways. Interestingly, sildenafil had a negative effect on pulmonary vascular development in healthy control fetal rats (26, 40) and rabbits (43).

In 2016, the first human study was initiated. The SToP PH Trial (EU Clinical Trials Register (2016–002619-17)) is a randomized controlled trial, investigator-blinded, double-armed, parallel group, phase I/IIb study designed to determine the safety profile of transplacental sildenafil delivery (44). The second arm of the study involving pregnant women with isolated CDH fetuses is currently on hold after significant treatment-related mortality was reported in a recent multicenter, international randomized controlled trial. The Dutch-STRIDER (NCT02277132) trial used antenatal sildenafil to treat early onset intrauterine fetal growth restriction, but was prematurely discontinued in 2018 following high mortality rates in the sildenafil group, including 11 newborn deaths (45). Previous human studies investigating antenatal sildenafil to manage pediatric PH (46), preeclampsia (47), and intrauterine fetal growth restriction (48, 49) found no severe maternal or fetal adverse events; however, when given at high prenatal doses, an increased risk of fetal toxicity and growth suppression was reported in fetal mice (50). These worrisome findings warrant careful consideration prior to clinical advancement.

Tadalafil: Tadalafil is a PDE inhibitor with greater PDE5 specificity and longer half-life than sildenafil (38, 51). Although predominantly used to treat adult PH, tadalafil has been used off-label for pediatric PH (34, 52). A surgical lamb model was used to investigate tadalafil for CDH-PH. Pregnant ewes underwent surgical diaphragmatic hernia creation at gestational day 75 (38), and then received oral tadalafil postoperatively for up to seven days. Treated fetal lambs had increased pulmonary vasodilation with improved pulmonary blood flow, and increased cGMP and eNOS levels. The upregulation of NO-cGMP pathway proteins likely contributed to tadalafil's ability to ameliorate vascular remodeling *in utero*. Of interest, tadalafil did not significantly improve oxygenation, pulmonary arterial pressures, or right ventricular hypertrophy. The lack of meaningful clinical changes may be due to

insufficient duration of treatment, which must be determined in further studies. No evidence of intrauterine fetal growth restriction or adverse fetal effects were observed. No human CDH studies exist, but positive results in animals and other forms of PH support conducting a trial in CDH patients (34, 52).

Phosphodiesterase type 3 (PDE3) has also been associated with pulmonary hypertension. Milrinone is a PDE3 inhibitor that is used in the neonatal population to treat persistent PH (53). Currently, there is an ongoing trial (NCT02951130) investigating the use of postnatal milrinone in CDH infants, which is planned to be completed in February 2021. While antenatal administration has yet to be explored, results from this study may support future investigations.

Soluble guanylate cyclase agonists

BAY 41–2272: BAY 41–2272 is a synthetic soluble guanylate cyclase stimulator that directly enhances receptor activity to promote cGMP-mediated vasodilation and anti-thrombotic activity, as well as reduced right ventricular systolic pressure and vascular remodeling in experimental PH models (54–56). In a fetal sheep model of persistent PH of the newborn, BAY 41–2272 improved pulmonary vasodilation and reduced pulmonary vascular resistance (57).

Rabbits with surgical diaphragmatic hernias received a single dose of BAY 41–2272 via tracheal instillation on E28 (58), and were retrieved at term. Results showed reduced right ventricular pressure and ameliorated vascular remodeling with decreased medial thickening in small arteries and increased capillary formation. Interestingly, in normal pathology, angiogenesis is predominantly regulated by VEGF-mediated activation of the NO-cGMP pathway. However, in this study, treated lungs had increased endothelial cell proliferation and vessel density with increased eNOS expression, but lacked VEGF overexpression. This suggests that BAY 41–2272's angiogenic effects are mediated via a VEGF-independent pathway, consistent with prior *in vitro* findings (59), and may present a novel therapeutic pathway. While larger studies are necessary, BAY 41–2272 seems promising with no evidence of maternal or fetal adverse effects.

BAY 60–2770: A soluble guanylate cyclase activator, BAY 60–2770, increases pulmonary vasodilation and reduces pulmonary and systemic arterial pressures in monocrotaline (MCT)-induced PH rats (60). To assess BAY 60–2770 in CDH-PH, neonatal pulmonary arteries were excised from fetal rabbits with surgical diaphragmatic hernias and studied *ex vivo* (61). Pulmonary arteries were harvested and bathed in phenylephrine solution to keep the vessels contracted. BAY 60–2770, tadalafil and the NO donor, sodium nitroprusside, were compared to determine effects on vasodilation. BAY 60–2770 induced potent vasorelaxation in the CDH group in a concentration-dependent manner, while tadalafil had no significant effect. *In vitro* analysis demonstrated increased levels of NO metabolites in the CDH group versus controls. The CDH group also had decreased arterial relaxation in response to sodium nitroprusside compared to controls, suggesting that reductions in soluble guanylate cyclase bioavailability and cGMP production may contribute to CDH pathology.

Therapies targeting cAMP pathways

The adenylate cyclase enzyme in pulmonary artery smooth muscle cells stimulates production of cyclic adenosine monophosphate (cAMP). This promotes vasodilation, anti-platelet aggregation, inhibition of inflammatory mediators, and regulation of smooth muscle cell proliferation and vascular development. Prostaglandins are vasodilatory compounds whose effects are mediated by the adenylate cyclase/cAMP pathway (62, 63). Within the prostaglandin family, prostacyclin is a particularly potent vasodilator that exhibits anti-inflammatory and anti-thrombotic properties. Patients with CDH-PH have decreased expression of the prostacyclin receptor (64), and small studies show that administration of prostaglandins improves symptoms, cardiac markers, echocardiography, and cardiac catheterization measurements which are all findings consistent with improved PH (65, 66). However, prostacyclin's efficacy is limited by a short half-life that requires continuous administration to achieve therapeutic levels (67). This creates substantial challenges as the risk associated with prolonged vascular access is significant. To address this limitation, synthetic prostacyclin analogues have been engineered and demonstrated variable success in PH.

Prostacyclin agonists

Selexipag: Selexipag (NS-304) and its active compound are oral, long-acting, selective prostacyclin receptor agonists (68). In adults with PH, selexipag reduced PH-related complications and death (69). Similarly, MCT-induced PH rats treated with selexipag demonstrated reduced right ventricular hypertrophy, arterial wall thickening, and improved survival (68). Mous *et al.* were the first to study targeting of dual vasodilatory pathways using selexipag and sildenafil in nitrofen-induced CDH rats (26). Treatment cohorts were divided into three groups: selexipag, sildenafil, or a combination of both therapies. Therapies were delivered daily via gastric lavage from E17.4 to E20.5, and fetuses were delivered at term. Isolated and/or combined therapy with selexipag and sildenafil decreased arterial wall thickening, smooth muscle cell proliferation, and right ventricular hypertrophy. Combined therapy also restored prostacyclin receptor expression to near-control levels. However, the combination of the two drugs did not exhibit any added therapeutic benefit, likely due to their competing hepatic metabolism. Administration of selexipag alone reduced PDE3 expression and the downstream target of PDE5, protein kinase G2, but did not impact eNOS. Thus, improvements in vascular remodeling may be partly due to normalization of key receptors within the prostacyclin pathway. No evidence of maternal or fetal adverse effects were identified. Notable, however, was an unexpected thickening of the medial layer in all treated control groups.

ONO-1301SR: ONO-1301 is a long-acting, synthetic prostacyclin agonist. In MCT-induced PH rats, serial ONO-1301 decreased pulmonary arterial wall thickening and mitigated right ventricular systolic pressure elevation (70). To improve drug delivery, a slow release formulation (ONO-1301SR) was created and showed comparable effectiveness (19, 71). Umeda *et al.* administered ONO-1301SR to nitrofen-exposed rats via a single, subcutaneous injection on E9.5 (19). Fetuses were retrieved at term. Results demonstrated positive effects on pulmonary hypoplasia, which we will not discuss here, as well as improved arterial remodeling evidenced by decreased arterial wall thickness and increased capillary

formation. Molecular studies found increased gene expression of VEGF, hepatocyte growth factor, and stromal cell-derived factor in the treated lungs. Protein levels of VEGF were also normalized with ONO-1301SR. Thus, upregulation of important growth factors likely promotes pro-angiogenic pathways to mitigate vascular remodeling. The authors also hypothesize that arterial wall thinning, as observed in the treatment group, may be due to modifications to the prostacyclin pathway versus an indirect result of the drug's anti-thrombotic properties. The exact mechanism remains unclear. Safety analysis following single and serial doses demonstrated no significant adverse effects; however, since antenatal ONO-1301SR is given early during organogenesis and is known to target multiple organs, an evaluation of off-target toxicity is necessary. This study performed a very limited assessment of accessory organs.

Therapies targeting endothelin pathway

Endothelin-1 (ET-1) is the primary ligand of the endothelin pathway and is a strong vasoconstrictor produced by the endothelin converting enzyme (72). ET-1 binds to two receptors to regulate vascular tone: endothelin receptor type A (ETA) induces vasoconstriction by promoting the release of cytosolic calcium within pulmonary artery smooth muscle cells, and endothelin receptor type B (ETB) primarily promotes vasodilation via upregulation of NO and prostacyclin (72). In nitrofen-induced pups, upregulation of ET-1 and both ET receptors is observed (23, 64, 73, 74), along with a heightened vasoconstrictive response to ET-1. CDH infants have elevated levels of ET-1 in plasma and lungs (75), and exhibit significant ET-1 receptor dysfunction with a more pronounced increase in ETA compared to ETB (64). The imbalance in ETA and ETB expression is primarily responsible for the shift in vascular tone towards persistent vasoconstriction.

Endothelin receptor antagonists

Bosentan: Bosentan is a dual ETA and ETB antagonist that increases vasodilation, reduces pulmonary vascular resistance, and has been shown to successfully treat newborn PH (76). To explore its role in CDH-PH, nitrofen-exposed rats were divided into three treatment groups: oral sildenafil, oral bosentan, or combined therapies. Treatments were administered via gastric lavage from E16 to E20 (41). Animals were harvested on E21. Bosentan alone did not improve vascular remodeling; however, CDH rats that received sildenafil or combined therapies demonstrated a significant reduction in medial wall thickening indicating attenuated vascular cellular proliferation. These data suggest that sildenafil is predominantly responsible for the changes observed in vascular remodeling.

Therapies targeting tyrosine kinase pathway

Receptor tyrosine kinases, such as platelet-derived growth factor (PDGF) receptors, have been strongly associated with pulmonary vascular remodeling (15). PDGF ligands and their associated receptors induce smooth muscle cell proliferation and migration, and regulate angiogenesis in experimental animal models (77). Elevated lung PDGF levels in PH patients indicate its likely involvement in the development of human disease (78).

Tyrosine kinase receptor inhibitors

Imatinib: Imatinib is a selective inhibitor of c-Kit and BCR-ABL tyrosine kinase receptors (79), including the PDGF receptor. Animal models and case reports of adults with severe PH demonstrate that imatinib effectively prevents and/or reduces pulmonary vascular pathophysiology (80, 81). A randomized controlled trial in adults with medically refractory PH found decreased pulmonary vascular resistance and increased cardiac output in imatinib-treated patients (82). Similarly, a case report of a CDH neonate with intractable PH showed decreased pulmonary arterial pressures and clinical improvement following imatinib administration (83).

Nitrofen-exposed rats received oral imatinib via gastric lavage from E17 to E20 (84). Fetuses were retrieved at term. Treated CDH lungs had improved vascular remodeling with a reduction in medial arterial wall thickness, number of fully muscularized arteries, vascular cell proliferation, and restoration of luminal area. Molecular analysis showed a trend toward downregulation of the PDGF-β ligand and its receptors, which normally promote smooth muscle cell proliferation (85), apoptosis, and vessel maturation (86). Contrarily, when this experiment was repeated by Burgos *et al.*, no significant therapeutic effect was observed in the treated group (87). This discrepancy may be attributable to different measurement criteria between the groups, but raises concerns about therapeutic efficacy.

Improvement in vascular remodeling in the treated rats likely corresponds to the inhibition of vascular cell proliferation via PDGF downregulation and induction of apoptosis in apoptotic-resistant smooth muscle cells (77, 84). Further studies are needed to understand the mechanism. Of concern is imatinib's known teratogenic effects at high doses, which were required to ameliorate vascular remodeling in preclinical studies (77). Although low doses in the nitrofen-exposed rats were largely safe, unanticipated arterial wall thinning and an increased number of muscularized vessels was found in the treated controls (84). Such abnormal findings within the therapeutic range are worrisome and mandate evaluation. Of interest, two other receptor tyrosine kinase inhibitors, nilotinib and dasatinib, showed positive *in vivo* results in this study. However, case reports of adults treated with dasatinib for chronic myeloid leukemia reported severe PH as a notable adverse effect (88, 89).

Therapies targeting pro-inflammatory pathways

Peroxisome proliferator-activated receptor-gamma (PPAR-γ) is a ligand-activated transcription factor involved in angiogenesis and pulmonary artery smooth muscle cell proliferation (90). PPAR-γ regulates inflammatory processes that induce vascular remodeling in many types of human and experimental PH models (91, 92). It inhibits expression of important inflammatory mediators, including monocyte chemoattractant protein-1 (MCP-1) (91–94) and interleukin 6 (IL-6) (93, 94). MCP-1 promotes monocyte perivascular infiltration, endothelial cell dysfunction, and smooth muscle cell proliferation (95–99). IL-6 is a pro-inflammatory cytokine highly associated with PH pathogenesis. Additionally, IL-6 is also activated by an upstream inflammatory cytokine known as the macrophage migration inhibitory factor, which further propagates the development of a chronic inflammatory immune response (100). Patients with PH have elevated plasma IL-6 (98, 101) and MCP-1 (102) levels, and in cases of severe disease, reduced PPAR-γ (103). In

CDH-PH, human and experimental models show increased pulmonary vascular levels of inflammatory markers (101, 104), including MCP-1 (96), while simultaneously demonstrating decreased levels of PPAR-γ (105). Thus, a heightened inflammatory state induces abnormal smooth muscle cell function and proliferation that contributes to vascular remodeling in CDH-PH.

PPARY-γ agonists

Rosiglitazone: Gosemann *et al.* investigated rosiglitazone, a PPAR-γ agonist, in a nitrofen-induced CDH rat model (97). Rats received daily intraperitoneal injections for two days (E18 to E19). Treated CDH fetuses harvested at term showed reduced arterial wall thickening, MCP-1 protein expression, and monocyte perivascular infiltration. The reduction in MCP-1 suggests that rosiglitazone's effect occurs via stimulation of the PPAR-γ pathway. However, the efficacy of MCP-1 in CDH-PH requires greater evaluation. The therapeutic safety profile of rosiglitazone was not assessed in this study.

Macrophage migration inhibitory factor inhibitors

ISO-92: ISO-92, a synthetic macrophage migration inhibitory factor inhibitor, was tested in nitrofen-induced rats (9). An osmotic device was implanted within the subcutaneous tissue of pregnant rats at E10 or E11, and continuously administered ISO-92 until term delivery. ISO-92 mitigated migration inhibitory factor activity but did not alter its expression or secretion. Treated mice had reduced medial wall thickness and lower right ventricular systolic pressure, suggestive of improved arterial remodeling and cardiovascular physiology, which are both essential to PH treatment. If applied clinically, failure to alter migration inhibitory factor expression may prove to be advantageous; since migration inhibitory factor is physiologically required for the newborn innate immune response, it is rational to suspect that downregulation of the cytokine may be potentially harmful. One downside with ISO-92 is that it is not widely available for use by other investigators or clinicians. Another obstacle is the drug's short half-life; thus, an invasive pump was required to continuously deliver therapy to the rodents. In the future, ISO-92 will need to be tested in a survival model to assess safety markers.

HMG-CoA reductase inhibitors

Simvastatin: Inhibitors of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase, known as statins, support endothelial cell function and promote vasorelaxation through upregulation and activation of eNOS, activation of phosphoinositide 3-kinase/Akt pathway (106), and inhibition of Rho GTPases (107, 108). Rho GTPases induce vasoconstriction in response to ET-1 stimulation (109). When used to treat patients with atherosclerotic disease, statins promote anti-inflammatory, anti-proliferative, and immunosuppressive properties that improve cardiovascular outcomes. In experimental PH models, simvastatin reduced neointimal hyperplasia, pulmonary arterial pressures, right ventricular hypertrophy, and effectively reversed PH (110, 111).

To investigate simvastatin in CDH-PH, nitrofen-exposed rats were randomized to three oral treatment arms: simvastatin, sildenafil, or placebo for ten days (E11 to E21) (23), and then harvested fetuses at term. Simvastatin-treated CDH fetuses had decreased medial wall

thickness in resistance level pulmonary arteries and a trend toward increased vascular density. Modifications in the endothelin pathway involved reduced gene expression of the ET-1 precursor, preproendothelin-1, and decreased ET-1 protein levels in treated lungs compared to the nitrofen-only group; furthermore, ETA gene expression was restored to control levels. Simvastatin-treated groups demonstrated a trend towards normalized iNOS and eNOS gene expression and higher NO lung levels compared to nitrofen-only groups. Additionally, simvastatin restored pro-apoptotic mechanisms evidenced by an increased ratio of Bax to Bcl-2. These findings suggest that simvastatin improves vascular remodeling by modulating the endothelin pathway, normalizing eNOS function and NO bioavailability, and promoting normal levels of smooth muscle cell apoptosis. The cumulative effect of restoring these pathways may help re-establish vasoreactivity, and normal function and phenotype of pulmonary artery smooth muscle cells. Notably, simvastatin-treated CDH rats had reduced body weight potentially signifying intrauterine fetal growth restriction, but morbidity related to growth retardation was not evaluated. Although human studies have shown safe administration in pregnancy (112), these findings warrant further investigation.

ANTENATAL REGENERATIVE THERAPIES FOR CDH-PH

Arrested lung and vascular development leads to pulmonary hypoplasia and PH in CDH infants. Regenerative therapies that can reverse these processes and enhance pulmonary development are desirable for management of CDH-PH. Mesenchymal stem cells (MSCs) have been investigated in many types of chronic lung diseases (113), and findings show that they inhibit inflammation, enhance immunity, and stimulate lung growth (113). In chronic hypoxia and MCT-induced PH experimental models, both intravascular and intra-tracheal MSC administration inhibited PH (114, 115). Angiogenic MSCs also restored alveolar and vascular morphology, and promoted lung development in experimental models of other neonatal pulmonary diseases (116).

In CDH, MSCs have been primarily explored to address lung hypoplasia (117), but it is rational to suspect that they may also be effective in CDH-related PH, as airway and vascular development are closely intertwined (118). Promising results in experimental models support this theory. Yuniartha *et al.* administered lung tissue MSCs from donor adult rats to nitrofen-exposed rats via a single uterine vein injection (119), and found that in addition to alleviating pulmonary hypoplasia, the treated CDH group also had reduced medial wall thickening compared to the nitrofen-only group. Similarly, Takayama *et al.* demonstrated decreased wall muscularization in nitrofen-exposed rats following intra-amniotic injection of human MSCs (120). An *ex vivo* study examined explanted nitrofen-exposed rat lungs, which were conditioned in amniotic fluid-derived MSC media, and found increased VEGF and fibroblast growth factor expression compared to controls (121). Though the exact mechanism is not fully understood, these findings suggest that therapeutic effects are likely due to MSC-mediated release of various paracrine factors including pro-angiogenic growth factors: VEGF, hepatocyte growth factor, fibroblast growth factor, and angiopoietin (117). Additional secretion of cytokines and chemoattractant factors may further induce regulatory mechanisms, which enhance vascular modifications. No significant maternal or fetal complications were reported in these studies. However, previous studies show that intravascular administration of MSCs is associated with immunodeficiency,

microvascular embolism, and inflammation (122, 123). To our knowledge, MSCs have never been explored to specifically address CDH-related PH, but given their success in experimental models, they certainly warrant further investigation.

Additionally, MSC-derived exosomes have also demonstrated promising findings when used to treat neonatal lung disease such as bronchopulmonary dysplasia (124). There is also evidence to suggest that exosomes are effective in treating vascular remodeling in other models of PH (125, 126). However, their application in CDH-specific PH has not been investigated. Given their previous success in both neonatal diseases and other forms of PH, exosomes present a likely suitable treatment approach that should be further explored.

CONCLUSION

Current therapies for CDH-PH are supportive and predominantly target vasodilation in the postnatal period. These interventions are likely too late as the disease is established in fetal development. Thus, development of novel antenatal therapies that address the pulmonary vascular remodeling before the disease progresses is logical. Amongst the emerging antenatal therapies discussed in this review, variable efficacy has been demonstrated in the attenuation of vascular remodeling. While many therapies show isolated therapeutic promise, no single agent effectively addresses the complex and multifactorial etiologies responsible for CDH-PH. The proposed antenatal therapies are largely non-specific, which poses an increased risk of systemic adverse effects both to the mother and fetus. Off-target organ injury in neonates could be particularly detrimental given the vulnerable period in which patients receive the therapy. Instead, the ideal therapeutic approach should use a drug delivery vehicle that specifically targets the diseased pulmonary vasculature and directly releases a therapeutic to the area of interest. Techniques such as regenerative stem cells or versatile nanoparticles, which are currently being investigated in other types of PH, should be strongly considered in the management of CDH-PH to further optimize antenatal options. Furthermore, a combination of synergistic therapies addressing the many molecular pathways involved in pulmonary vascular remodeling will likely be necessary to effectively mitigate the pulmonary hypertension associated with congenital diaphragmatic hernia.

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Impact

- Emerging antenatal therapies for congenital diaphragmatic hernia-induced pulmonary hypertension (CDH-PH) show promise to effectively mitigate vascular remodeling in preclinical models. Further investigation is needed in preclinical and human studies to evaluate safety and efficacy prior to translation into the clinical arena.
- This review offers a comprehensive and up-to-date summary of emerging therapies currently under investigation in experimental animal models.
- There is no cure for CDH-PH. This review explores emerging therapeutic options for the treatment of CDH-PH and evaluates their impact on key molecular pathways and clinical markers of disease to determine efficacy in the preclinical stage.

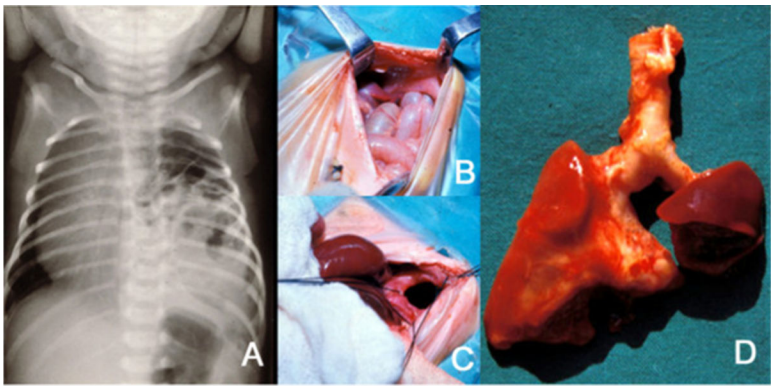

Figure 1. CDH infant with impaired lung development.

(A) Chest X-ray of infant with congenital diaphragmatic hernia. Intraoperative image of diaphragmatic defect with (B) herniated bowel and (C) subsequent reduction back into the abdomen. (D) Gross image of severe ipsilateral lung hypoplasia. Images reprinted from Tovar *et al.* (127) under the terms of the Creative Commons CC BY license.

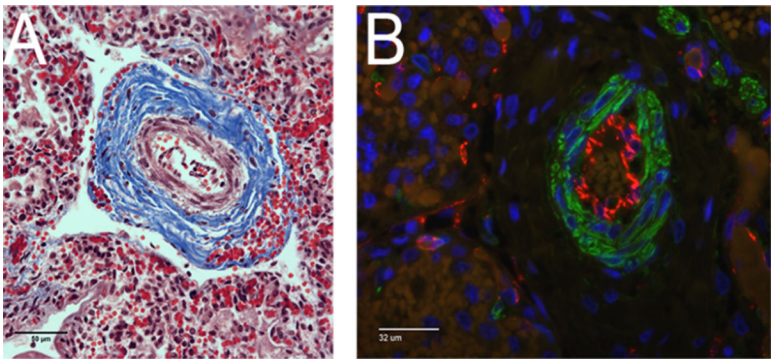

Figure 2.

CDH infant with pulmonary hypertension. (A) Masson's Trichrome staining demonstrating increased wall muscularization in pulmonary arterioles. (B) Immunofluorescence staining for smooth muscle-alpha actin (green) demonstrates increased pulmonary arterial smooth muscle cell proliferation. Images acquired at 40x.

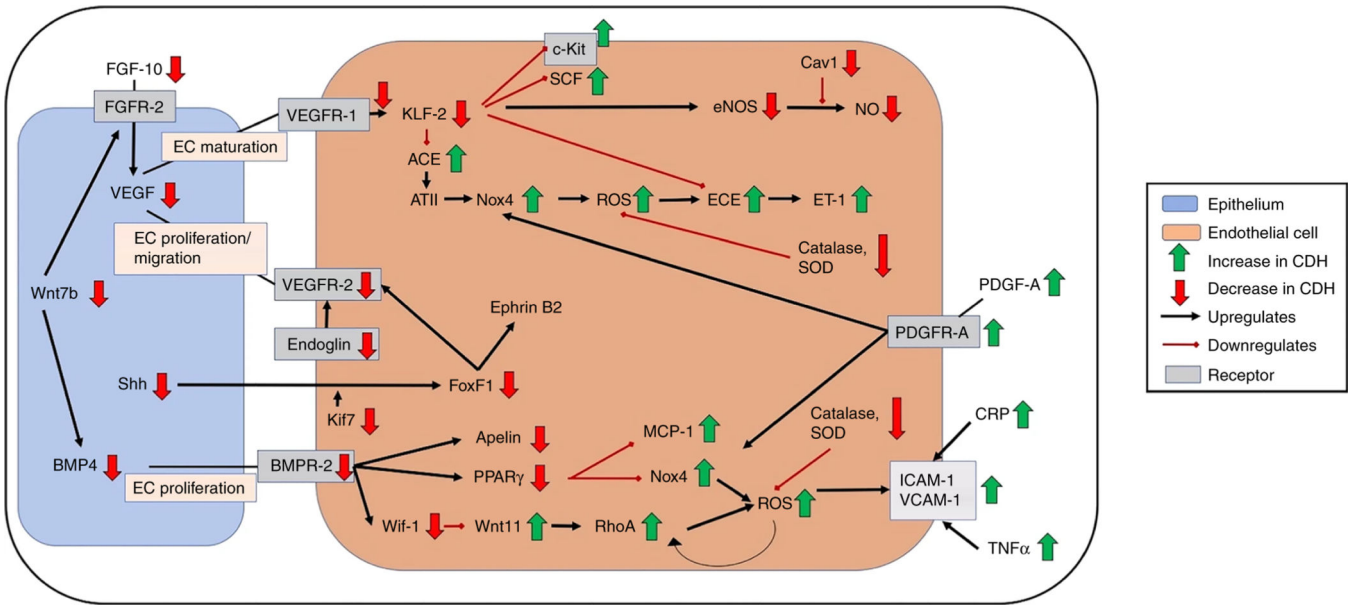

Figure 3.

Molecular pathways involved in vascular remodeling in congenital diaphragmatic hernia-induced pulmonary hypertension. Reprinted by permission from Springer Nature Customer Service Centre GmbH: Nature. Pediatric Research; 85,754–768. Pulmonary hypertension secondary to congenital diaphragmatic hernia: factors and pathways involved in pulmonary vascular remodeling, Louise Montalva *et al*. COPYRIGHT (2019) (15).

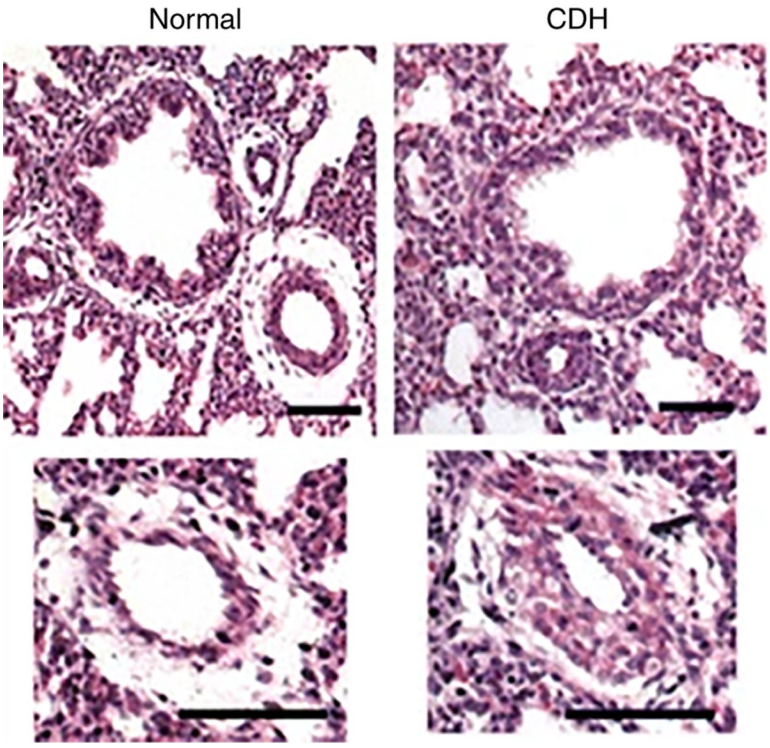

Figure 4.

Representative images of hematoxylin and eosin staining in lung tissue of control versus nitrofen-induced CDH rat lungs. Scale bars: 100μm. Reprinted by permission from Springer Nature Customer Service Centre GmbH: Springer. Pediatric Surgery International; 29(8):823–834. Downregulated bone morphogenetic protein signaling in nitrofen-induced congenital diaphragmatic hernia, Martine Makanga *et al.* COPYRIGHT (2013) (128).

Table 1.

Effects of emerging antenatal pharmacotherapies on vascular remodeling in CDH-PH animal models.

Drug class	Drug name	Animal model	Molecular changes	Effect on pulmonary vasculature
Phosphodiesterase inhibitors	Sildenafil	Nitrofen-induced rats(22, 23, 26, 40–42, 129, 130) Surgical diaphragmatic hernia rabbits(43)	↑ NO(23), iNOS(23), eNOS(22, 23), cGMP(22) ↓ ET-1(23), ETA(23), PPET-1(23), ECE-1(23) ↑ VEGF(22) ↓ PDE5 (active)(22), Prkg2(26)	↑ Vasoreactivity to NO(22) and VEGF(43) ↑ Vasorelaxation(22, 43, 130) ↑ Vessel density(22, 23, 42, 43) ↓ Vessel wall muscularization(22, 23, 26, 40–43)
	Tadalafil	Surgical diaphragmatic hernia ewes(38)	↑ eNOS ↑ cGMP No effect on PDE5 levels	↑ Vasodilation ↑ Total blood flow
		Ex vivo fetal rabbit pulmonary arteries(61)	N/A	No effect on vasodilation
Endothelin receptor antagonists	Bosentan	Nitrofen-induced rats(41)	N/A	No effect on vessel wall thickness
Soluble guanylyl cyclase agonists	BAY 41-2272	Surgical diaphragmatic hernia rabbits(58)	↑ eNOS No effect on ET-1 or VEGF	↓ Muscularization in small arteries ↑ EC proliferation & capillary formation
	BAY 60-2770	*Ex vivo* fetal rabbit pulmonary arteries(61)	N/A	↑ Vasodilation
Prostacyclin agonists	Selexipag	Nitrofen-induced rats(26)	↓ PDE-3 and Prkg2 No change in eNOS or PDE5	↓ Vessel wall muscularization No effect on vessel branching or total volume
	ONO-1301SR	Nitrofen-induced rats(19)	↑ VEGF	↓ Medial wall thickness ↑ Vascular bed formation
Tyrosine kinase receptor inhibitors	Imatinib	Nitrofen-induced rats(84)	↓ PDGF-β ligand and receptors	↓ Medial wall thickness ↓ Percentage of fully muscularized arteries ↓ Vascular cell proliferation
HMG-CoA reductase inhibitors	Simvastatin	Nitrofen-induced rats(23)	↓ PPET1, ET-1, ETA, ETB, ECE-1 ↑ iNOS, eNOS and NO ↑ Pro-apoptotic mechanisms	↓ Medial wall thickening ↑ Vessel density
PPAR-γ agonists	Rosiglitazone	Nitrofen-induced rats(97)	↓ MCP-1 protein levels ↓ Monocyte perivascular infiltration	↓ Arterial wall thickening
Macrophage migration inhibitory factor inhibitors	ISO-92	Nitrofen-induced rats(9)	↑ eNOS ↑ VEGF	↓ Medial wall thickness

Abbreviations: PDE5, phosphodiesterase type 5; NO, nitric oxide; iNOS, inducible nitric oxide synthase; eNOS, endothelial nitric oxide synthase; cGMP, cyclic guanosine monophosphate; ET-1, endothelin-1; ETA, endothelin receptor type A; PPET-1, preproendothelin-1; ECE-1, endothelin converting enzyme; VEGF, vascular endothelial growth factor; Prkg2, protein kinase G2; EC, endothelial cell; PDE3, phosphodiesterase type 3; PDGF, platelet-derived growth factor; ETB, endothelin receptor type B; HMG-CoA, 3-hydroxy-3-methyl-glutaryl-coenzyme A; PPAR-γ, peroxisome proliferator-activated receptor-γ; MCP-1, monocyte chemoattractant protein-1