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Review

Understanding and treating pulmonary hypertension in congenital diaphragmatic hernia

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SUMMARY

Lung hypoplasia and pulmonary hypertension are classical features of congenital diaphragmatic hernia (CDH) and represent the main determinants of survival. The mechanisms leading to pulmonary hypertension in this malformation are still poorly understood, but may combine altered vasoreactivity, pulmonary artery remodeling, and a hypoplastic pulmonary vascular bed. Efforts have been directed at correcting the "reversible" component of pulmonary hypertension of CDH. However, pulmonary hypertension in CDH is often refractory to pulmonary vasodilators. A new emerging pattern of late (months after birth) and chronic (months to years after birth) pulmonary hypertension are described in CDH survivors. The true incidence and implications for outcome and management need to be confirmed by follow-up studies from referral centers with high patient output. In order to develop more efficient strategies to treat pulmonary hypertension and improve survival in most severe cases, the ultimate therapeutic goal would be to promote lung and vascular growth.

1. Introduction

Congenital diaphragmatic hernia (CDH) is a defect of the diaphragm allowing the abdominal content to ascend into the thorax. CDH has an overall birth prevalence rate of 3.5 per 10,000, of which 69% result in live births [1]. Survival at one year can be as high as 77% of live births in infants with isolated CDH. However, survival at one year decreases to 42% when considering all infants prenatally diagnosed with CDH [1]. Pulmonary hypertension and lung hypoplasia are constant findings in CDH and represent the two main factors determining the outcome [2]. Because of its peculiar pathophysiology, pulmonary hypertension in CDH is often resistant to standard vasodilator treatments. Although the mechanisms underlying refractory pulmonary hypertension in CDH are not fully understood, it is believed that altered vasoreactivity in combination with pulmonary vascular remodeling is superimposed to varying degrees of pulmonary vascular bed hypoplasia, which may account for the extremely challenging management of this form of pulmonary hypertension of the newborn [2]. This review analyzes the current knowledge on the causes of the pulmonary hypertension of CDH and the current practice and the future options for its treatment.

2. Pathophysiology of pulmonary hypoplasia and pulmonary hypertension in CDH

2.1. Lung hypoplasia and delayed lung development represent the hallmark of CDH

The classical pulmonary histological findings of CDH consist of an arrest in alveolar development with fewer alveoli and reduced gas-exchange surface area, markedly thickened alveolar walls, and increased interstitial tissue [3]. Although more pronounced in the ipsilateral lung to the CDH, the same features can be recognized in both lungs [4]. Lung hypoplasia in CDH was initially ascribed to the effect of the visceral organs herniated into the thorax, resulting in lung compression and arrest in lung development. The time frame was believed to start at about eight or nine weeks of gestation, after the physiological closure of the diaphragm and the establishment of the separation between abdominal and thoracic organs [5]. More recently, a new insight arose from a murine model, in which CDH is induced in the offspring by maternal ingestion of the herbicide, nitrofen, during early pregnancy [6]. The histological studies showed that the lung hypoplasia and the arrest in lung development occur before the normal closure of the diaphragm and they

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are, therefore, at least in part, independent from the mechanical compression of the ascended abdominal organs [7]. These discoveries led to the formulation of the "dual hit" hypothesis [8]. The first hit, possibly genetic or environmental, would occur at an early stage, before the development of the diaphragm, and it would arrest lung development in both lungs. The lung compression by the abdominal organs at a later phase (second hit) would then worsen the ipsilateral hypoplasia, explaining why both lungs are usually hypoplastic, but the ipsilateral lung is more affected than the contralateral [8].

2.1.1. Retinoid signaling and lung growth in CDH

It has been suggested that early lung hypoplasia and disruption of diaphragm closure may be caused by a perturbation of the retinoid signaling pathway, which is known to play an important role in normal lung development [9]. In humans, CDH has been associated with low maternal vitamin A intake during pregnancy [10] and low levels of retinoids at birth [11]. Moreover, large doses of vitamin A administered during gestation can reduce the incidence of nitrofen-induced CDH by 15–30% and attenuate lung hypoplasia in rats [9].

2.1.2. Vascular growth factors and lung development in CDH

Evidence suggests that angiogenesis contributes to normal lung growth [12]. The most studied molecule has been vascular endothelial growth factor (VEGF), an endothelial cell-specific mitogen and survival factor that stimulates angiogenesis and protects from endothelial cell injury. VEGF stimulates NO production in cultured cells and isolated blood vessels. At the same time, NO is essential in mediating the VEGF-induced endothelial cell proliferation [13]. In animal models, VEGF was shown to play a critical role in lung vascular and epithelial development [14]. Although the link is still unclear, VEGF may take part also in the CDH vascular underdevelopment. The expression of VEGF in the nitrofen-induced CDH was found to be either increased [15] or decreased [16,17]. In humans, VEGF expression is increased in the lungs of infants who died from CDH [18] and fetuses after medical termination of pregnancy due to prenatal diagnosis of CDH [19]. During fetal life, the VEGF increase matched with a decreased eNOS expression, detected from the end of the canalicular stage (22–25 weeks). The increase in VEGF expression could be interpreted as an attempt of the hypoplastic lung to overcome the vascular underdevelopment, possibly caused by the reduced NO production, attributable to the decrease eNOS expression.

2.1.3. Role of endothelial progenitor cells

Endothelial colony-forming cells (ECFCs), a subset of circulating and resident endothelial cells capable of self-renewal and de-novo vessel formation, are thought to contribute to vasculogenesis in the developing lung. ECFCs are markedly decreased in cord blood of extremely premature infants, especially those who will develop moderate or severe bronchopulmonary dysplasia, the chronic lung disease of prematurity [20]. On the contrary, ECFCs are more numerous in the cord blood of CDH infants and can proliferate significantly more rapidly, compared to ECFCs from healthy patients [21]. It is possible that, in CDH, ECFC overproduction is one of the response mechanisms to the impaired vascular development, whereas the failure to put in place this mechanism, particularly weak in extremely preterm infants, may predispose to bronchopulmonary dysplasia. Interestingly, a population of lung resident highly proliferative endothelial cells (HP-PAECs) obtained from pulmonary arteries of sheep with surgical-induced CDH, was decreased in number and impaired in function (defective growth and tube formation in vitro) compared to HP-PAECs from control animals. HP-PAECs from fetal sheep with CDH also had increased levels of VEGF and decreased eNOS protein and NO production, compared to HP-PAECs from control animals. Exogenous VEGF and NO were able to restore PAEC function [22]. These data suggest that HP-PAEC dysfunction may partially be responsible for the altered angiogenesis of CDH, due to the VEGF-NO signaling pathway impairment.

These observations have potential therapeutic implications since exogenous human cord blood-derived ECFCs restore normal lung alveolar and vascular growth, diminish pulmonary hypertension and restore resident lung ECFC function in another animal model of altered lung vascular growth [23]. Understanding the role of progenitor cells in the pathogenesis of the pulmonary hypertension of CDH may pave the way to new therapeutic strategies by enhancing the physiological protective mechanisms of the lung.

2.2. Pulmonary vascular remodeling contributes to structural, "irreversible" pulmonary hypertension in CDH

Along with pulmonary hypoplasia, varying degrees of pulmonary hypertension are a constant finding in CDH. Structural changes of the lung vasculature, with reduced size of the pulmonary vascular bed, have been documented in CDH patients. The pulmonary arteries are hypertrophied [24] and composed of more abundant contractile vascular smooth muscle cells, disposed more distally than normal; the media and adventitia are thickened [25]. These findings of underdevelopment and hypertrophy of the vascular bed are regarded as the "fixed" and "irreversible" component of pulmonary hypertension in CDH.

2.3. Altered vasoreactivity

A "reversible" hyperresponsiveness of pulmonary arteries may contribute to pulmonary hypertension in CDH [26]. Analysis of human lung specimens, obtained from neonates who died from CDH, showed reduced and abnormal innervation of the peripheral airways, with increased sympathetic and decreased parasympathetic tone. The imbalance of autonomic innervation could be a contributing factor to the pulmonary hypertension and hyperreactivity in infants with CDH [27]. Animal studies showed increased contractility and impairment in endothelium-dependent relaxation of pulmonary arteries in CDH [28,29]. The contribution of this increased "reversible" vasoreactivity is variable. In clinical practice, different and sometimes absent responses to vasodilator therapies are seen, probably depending on the degree of vascular underdevelopment, which remains difficult to evaluate.

Various molecules and pathways have been investigated in order to understand the pathogenetic mechanisms involved in the pulmonary hypertension of CDH. Endothelin-1 (ET-1), a protein synthesized by endothelial cells, is a potent vasoconstrictor. ET-1 is known to play a crucial role in the development of the persistent pulmonary hypertension of the newborn (PPHN) and it has been proposed as a possible candidate in the establishment of pulmonary hypertension of CDH. ET-1 exerts its effects through two distinct receptor subtypes: the ET-A receptor, which is localized on vascular smooth muscle cells and induces vasoconstriction, and the ET-B receptor, which is mainly localized on the vascular endothelial cells and usually induces vasodilatation. Loss of ET-B receptor-mediated pulmonary vasodilation, increased lung ET-1 content, and increased ET-A receptor-mediated pulmonary vasoconstriction have been documented in animal models of PPHN and CDH [30–33]. In patients with CDH, dysregulation of the ET receptor balance also occurs. Both ET receptors are upregulated in the pulmonary arteries of CDH patients, although ET-A increase is more pronounced [34]. Keller et al. found that ET-1 plasma levels at two weeks of life are significantly higher in infants with CDH who will