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COMMENTARY

Tracheal Ligation and Corticosteroids in Congenital Diaphragmatic Hernia: For Better for Worse?

Commentary on the article by Kay *et al.* on page 495

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Congenital diaphragmatic hernia (CDH) continues to frustrate clinicians for several reasons: it is not possible to predict accurately the extent of pulmonary hypoplasia in the individual fetus and the postnatal response to treatment modalities such as inhalational nitric oxide (NO) is variable. Moreover, the use of extra corporeal membrane oxygenation (ECMO) carries a high morbidity and sometimes profound long-term sequelae.

Analysis of the literature reveals a myriad of treatment modalities proposed as "solutions" for the problem pulmonary hypoplasia in CDH. They range from termination of pregnancy following prenatal ultrasound to gentle handling of the lung postnatally to diminish iatrogenic damage of the fragile hypoplastic lungs (1). The fetal sheep model of CDH opened the way to fetal intervention and endoscopic fetoscopic procedures (FETENDO) such as tracheal ligation/occlusion in the human (2–7). Others have studied the basic mechanisms of pulmonary growth in a drug-induced CDH model (the Nitrofen rodent model) with or without the evaluation of additional treatment modalities such as corticosteroids and/or TSH releasing hormone (TRH) (8–14).

The supposed "lung growth" resulting from tracheal ligation is not founded on clean concepts of underlying mechanisms, although a variety of factors has been suggested (15–17).

Intriguing are recent observations from different laboratories of the negative effects of tracheal ligation, in fetal sheep, on type II cell differentiation, as nicely evaluated by Kay *et al.* in this issue (18). The question of whether we will end up with a combined approach of tracheal ligation/occlusion and maternal betamethasone therapy to rescue type II cell differentiation as optimal treatment of CDH in humans is therefore still open.

We have to bear in mind that the experiments described in the paper of Kay *et al.* were not conducted in a CDH model. As a consequence, we can only assume that the response will be the same in the hypoplastic lungs of CDH. However, no research data are available to support this assumption. Other questions are unanswered too, because the authors only assessed certain structural features of Type II cell density and markers of mRNA for two surfactant proteins. Whether steroids do more than up-regulate mRNA for the surfactant proteins or enhance function remains controversial. The study by Kay *et al.* involved no physiologic assessment of lung function, gas exchange or the development of pulmonary hypertension.

Before we apply the results of the experimental approach of Kay *et al.* in a clinical setting, we have to analyze the arguments for the use of corticosteroids to enhance lung development in prenatally diagnosed CDH, because the few reports on the use of corticosteroids in human CDH consist of personal communications, individual case reports, and anecdotal small series. However prenatal steroids are used to enhance lung development in premature infants.

A meta-analysis of published studies on prenatal glucocorticoids in threatened premature labor of fetuses without CDH consistently demonstrated beneficial effects on neonatal outcome for those infants born at 24 to 34 wk of gestation (19). Therefore, the National Institutes of Health consensus recommends antenatal administration of corticosteroids at least for 24 h, but if possible for 48 h, to all fetuses between 24 and 34 wk of gestation at risk of preterm delivery (20). However, as often suggested, it is still contradictory whether lungs of animal and human infants with CDH are surfactant deficient and morphologic immature like lungs from premature infants with surfactant deficient lung disease that do benefit from antenatal corticosteroid therapy (21). As a consequence it is hard to predict the significance of antenatal corticosteroids for the individual CDH patient. Moreover, we still do not know the long-term effects of antenatal corticosteroid treatment and there is growing evidence that these drugs may have adverse perinatal and longer term effects (22, 23). Profound effects on postnatal alveolar septation have already been documented (24, 25). In this light another important issue, recently raised by Smith *et al.* (22), is whether to use multiple or single antenatal courses of corticosteroids which is currently in use.

Considering the high incidence of chronic lung disease in CDH infants (26) the same concern is warranted for postnatal use of corticosteroids as recently eluded upon by Stark *et al.* in another group of patients: extremely-low-birth-weight infants (27).

We have to await the result of a recently started randomized controlled trial evaluating the use of betamethasone in prenatally diagnosed CDH, guided by the International CDH study group (principal investigator: Dr. K. Lally, Houston, U.S.A.)

After demonstration of accelerated lung maturation in premature sheep by Liggins (28, 29), several experimental studies in the Nitrofen CDH rodent model as well as in the surgically created sheep model have shown the beneficial effects of antenatal corticosteroid therapy including acceleration of the synthesis and release of surfactant, reduction of alveolar septal thickness, increase in maximum lung volume and compliance, and improvement in the antioxidant defense mechanisms (8–14). Prenatal use of corticosteroids in the Nitrofen model supposedly changes the pulmonary vascular architecture, although there are no reports of the expression of the glucocorticoid receptor in small pulmonary arteries. In contrast, expression of the thyroid hormone receptor has been described (30). The administration of glucocorticoids and TRH to Nitrofen-treated pregnant rats increased di-saturated phosphatidylcholine levels in the fetal offspring, reduced lung glycogen levels and significantly improved lung compliance and morphology (8–11). In addition, in our CDH rat model the combination of dexamethasone and TRH treatment of pregnant rats did not affect survival during ventilation in the pups and decreased glutathione reductase (14). We, therefore, concluded that antenatal administration of dexamethasone as a monotherapy would offer better prospects for randomized trials in prenatally diagnosed children with CDH than would the combination of dexamethasone and TRH.

Mechanisms of Glucocorticoid Action

During fetal and postnatal development, glucocorticoids function as signaling molecules to modulate the orderly sequence of differentiation in most tissues. From midgestation onwards, the fetus is exposed to increasing levels of cortisol of primarily fetal origin. Many studies, in humans as well as in animals, have provided strong evidence that the administration of glucocorticoids to the immature fetus accelerates lung maturation. Even "physiologic stressors." such as infection and premature rupture of the membranes, have been shown to accelerate fetal lung maturation, indicating that endogenous fetal glucocorticoids are instrumental in the normal course of lung maturation too. Endogenous hormones, however, do not initiate alveolar epithelial maturation, but are only involved in the modulation of genes responsible for surfactant production (for review see ref. 31).

Corticosteroids are known to induce several components of surfactant and to increase saturated phosphatidylcholine by stimulating key enzymes involved in phospholipid synthesis, such as fatty acid synthetase, choline phosphate cytidylyltransferase, and lysophosphatidylcholine acyl CoA acyl transferase. In addition, they stimulate lamellar body development in type II cells, and increase both tissue and alveolar content of surfactant (32). Finally, they also increase levels of the surfactant-associated proteins A, B, C, and D. In addition to these positive effects on surfactant production, glucocorticoids also stimulate antioxidant enzyme activity (33). Although still not completely defined, some of these effects appear to result largely from increased production of fibroblast-pneumonocyte factor by the fetal lung fibroblasts (34).

In contrast to these positive effects of corticosteroids, a variety of negative effects have been documented in the literature. In cultured lymphocytes, glucocorticoids caused apoptosis and an arrest in the G1 phase of the cell cycle thereby affecting proliferation which may finally lead to a reduction in cell number (35).

Also, prenatal dexamethasone treatment reduced overall DNA, but not the collagen content in lung tissue of neonatal rats (36). Moreover, a recent study showed that prenatal administration of dexamethasone to premature rats exposed to prolonged hyperoxia resulted in increased fibrosis in the dexamethasone treated lungs compared with the lungs from untreated animals (37).

In embryonic rat lung studies, corticosteroid treatment causes distorted branching, tubular dilatation, suppression of lung growth and epithelial cell proliferation, attenuation of mesenchymal tissue and compression of mesenchyme between adjacent epithelial tubules which represent the features of both distorted and accelerated maturation (38, 39).

Glucocorticoid-Receptor Interaction

Glucocorticoids exert their effects *via* a nuclear receptor of the steroid hormone receptor superfamily. This superfamily includes a number of ligand-responsive transcriptional enhanced proteins, including the glucocorticoid and thyroid hormone receptors. All members of the family share a highly conserved modular structure, with discrete functional domains for hormone binding, DNA binding, and transactivation (40). Concomitant with a rise in glucocorticoid plasma levels near term, enhancement of glucocorticoid receptor gene expression has been shown by Sweezy *et al.* in the fetal rat (41). Autoradiographic localization studies demonstrated increased glucocorticoid receptor gene expression in the mesenchyme, and more specifically in those mesenchymal cells adjacent to the terminal saccular epithelium (the cell population responsible for fibroblast-pneumonocyte factor production) (42, 43). To enhance our understanding of the potential effects of corticosteroids on the hypoplastic lung, we studied the glucocorticoid (GC)-receptor in hypoplastic CDH rat lungs and age-matched controls. No significant differences were observed in the tissue distribution or time of appearance of the GC-receptor under these experimental conditions (30).

Modulation of Pulmonary Growth in CDH

Findings presented at the 16th annual ECMO meeting in Keystone (March 2000) by investigators from Boston (J. Wilson, J. Schnitzer) and Liverpool (P. Losty) made it likely that members of the fibroblast growth factor family play important roles in an organotypic culture system of hypoplastic lungs in Nitrofen-induced CDH. During that same meeting Ch. Stolar

and colleagues advocated a pivotal impact of other well known growth factors, such as vascular endothelial derived growth factor (VEGF), on lung growth following tracheal ligation in the sheep CDH model as well as in an organotypic culture system including both the embryonic rat heart and lung buds. This is especially intriguing because human CDH lungs showed abnormal expression of VEGF, even in the endothelium of pulmonary arteries less than 75 μm (44).

In the light of the international corticosteroid treatment protocol of prenatally diagnosed CDH, including over 300 patients, and the National Institutes of Health sponsored tracheal ligation/occlusion study of prenatally diagnosed CDH patients with the highest risk ("liver up" patients), we must bear in mind that our knowledge of the optimal way to modulate prenatal pulmonary growth and differentiation is far from complete.

Although corticosteroids are known to induce apoptosis, they exert intriguing effects, not yet evaluated in humans, on VEGF expression and platelet-derived growth factor (PDGF), and on the PDGF-A receptor and pulmonary fibroblasts (45–47).

The presumed negative effects of tracheal ligation on type II cell differentiation in experimental CDH models are reason to carefully evaluate the use of corticosteroids in human cases. Not only the overall outcome should be assessed but also the more fundamental cell-biologic changes occurring during the transition from the saccular phase of pulmonary development to the alveolar phase, which takes place late in gestation in humans. The application of new technology such as the use of micro-arrays will help us to understand pulmonary development at the molecular level. The time dependent expression of a number of genes relevant for the progression in lung development in general (48, 49) should be taken into account, both in spontaneous as well as in experimental induced CDH. In this way we will be able to pinpoint the exact mechanisms resulting into pulmonary hypoplasia in CDH as well as the effect of modulating "agents" such as corticosteroids. We cannot run the risk that tracheal ligation with or without corticosteroids although "again" suggested as magic bullets for the improvement of the survival rate in newborns with CDH, turns out to be a new chapter in the book of unanswered questions in congenital diaphragmatic hernia.

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