Temporary tracheal occlusion in fetal sheep with lung hypoplasia does not improve postnatal lung function

MARCUS G. DAVEY, HOLLY L. HEDRICK, SARAH BOUCHARD, JULIANNE M. MENDOZA, UWE SCHWARZ, N. SCOTT ADZICK, AND ALAN W. FLAKE

Children’s Institute for Surgical Science and Center for Fetal Diagnosis and Treatment, Children’s Hospital of Philadelphia, Philadelphia, Pennsylvania 19104-4399

Submitted 7 August 2002; accepted in final form 7 November 2002

Davey, Marcus G., Holly L. Hedrick, Sarah Bouchard, Julianne M. Mendoza, Uwe Schwarz, N. Scott Adzick, and Alan W. Flake. Temporary tracheal occlusion (TO) in fetal sheep with lung hypoplasia does not improve postnatal lung function. J Appl Physiol 94: 1054–1062, 2003; 10.1152/japplphysiol.00733.2002.—Prolonged fetal tracheal occlusion (TO) accelerates lung growth but leads to loss of surfactant protein mRNA expression. Diaphragmatic hernia (DH) was created in 22 fetal sheep at 65 days of gestation. TO was performed between 110 days of gestation and full term (DH/TO, n = 7) and between 110 and 130 days of gestation (DH/TO + R, n = 6). Sham-operated fetuses (n = 11) served as controls. Lambs were delivered at ~139 days of gestation, and blood gas tensions were monitored over a 2-h resuscitation period. Temporary TO increased growth of the hypoplastic lung and restored surfactant protein mRNA expression and AE2 cell density but did not improve respiratory function above that of animals that underwent prolonged TO; DH/TO and DH/TO + R lambs were hypoxic and hypercapnic compared with Sham animals. Lung compliance remained low in DH/TO + R lambs, most likely as a consequence of the persistent increase in alveolar wall thickness in these animals.

Surfactant; lung growth; fetus

Severe congenital diaphragmatic hernia (DH) is associated with high postnatal morbidity and mortality due to a combination of reduced surface area available for the diffusion of respiratory gases (20) and impaired pulmonary perfusion secondary to pulmonary hypertension (23). Postnatal treatment of infants with pulmonary hypoplasia, including extracorporeal membrane oxygenation, inhaled nitric oxide therapy, and ventilator strategies that minimize ventilator-induced lung injury, may often be of minimal benefit to infants with severe congenital DH due to underlying gross deficits in pulmonary tissue. Recently, there has been great interest in prenatal correction of lung hypoplasia in human fetuses predicted to have poor postnatal outcome by in utero tracheal occlusion (TO) (12, 16). It is well established from animal studies that occluding the fetal trachea prevents the normal egress of liquid from the lung, leading to increased levels of lung tissue stretch and accelerated pulmonary growth.

Experiments performed primarily in fetal sheep have demonstrated that prolonged TO (>2 wk) leads to an almost total loss of the surfactant-producing alveolar epithelial type II (AE2) cells (4, 13, 14). During fetal lung development, AE2 cells are thought to act as precursors for AE1 cells (32). Furthermore, the differentiation of AE2 cells into AE1 cells is strongly influenced by local distending forces within the lung, such that increased levels of lung tissue stretch accelerate the differentiation of AE2 cells to AE1 cells (4, 13, 14). Consequently, the lungs of animals that have undergone prolonged fetal TO are “stiff” and unable to function effectively in respiratory gas exchange (25). Administration of exogenous surfactant to lambs that have undergone prolonged TO to reverse an existing lung hypoplasia has been shown to improve postnatal respiratory gas exchange (25). These data suggest that restoring normal levels of pulmonary surfactant may be pivotal in achieving normal postnatal lung function after periods of prolonged TO.

It is now evident that restoring the normal egress of liquid from the lung after a period of in utero TO (i.e., temporary TO) leads to the recovery of AE2 cells and their ability to produce surfactant (5, 7, 11, 14, 17, 21). Because administration of exogenous surfactant markedly improves postnatal lung function after prolonged TO (25), we hypothesized that temporary TO in fetuses with severe lung hypoplasia would 1) increase the surface area available for respiratory gas exchange at birth, 2) lead to recovery of AE2 cell density and surfactant protein (SP) mRNA expression, and 3) improve postnatal lung function above that of animals that underwent prolonged TO.

In this study, we examined the effects of temporary TO in fetal sheep with severe lung hypoplasia on early neonatal respiratory function, lung tissue structure, and SP gene expression. Lung hypoplasia was induced...
by surgical creation of DH at ~65 days of gestation in fetal sheep (full term ~145 days), which resulted in a >60% reduction in dry lung weight-to-body weight ratio at full term. Fetal TO was performed at 110 days of gestation and was maintained for 3 wk (temporary TO) or 4 wk (prolonged TO). Lambs were delivered near term (138–139 days of gestation) and mechanically ventilated for 2 h, during which we assessed the capacity of the lung to exchange respiratory gases. Pulmonary compliance curves were also obtained, so that we could correlate differences in postnatal pulmonary function with measurements of lung tissue structure (e.g., septal wall thickness and AE2 cell density) and SP gene expression. Because fetal surgery in sheep influences fetal pulmonary development (33), a group of lambs that underwent Sham surgical procedures at 65 and 110 days of gestation were also studied.

MATERIALS AND METHODS

All experimental protocols were approved by the Institutional Animal Care and Use Committee at the Children’s Hospital of Philadelphia and followed guidelines set forth by the National Institutes of Health.

Surgical creation of fetal DH. Thirty-three time-dated pregnant Western Cross ewes underwent aseptic surgery at 64–69 days of gestation (full term ~145 days). Ewes were sedated with ketamine (20 mg/kg im), and anesthesia was induced and maintained with isoflurane in O2. A midline laparotomy was performed, and the lower torso of the fetus, up to the level of the umbilical cord, was exposed via a small hysterotomy. A left-sided posterolateral thoracotomy was made between the second and third lower ribs to allow access to the fetal diaphragm. In 22 of 33 fetuses, the diaphragm was incised, and at least two stomachs were positioned in the thorax. In the remaining 11 fetuses, the diaphragm was left intact; these animals served as control (Sham) animals. The thoracotomy was closed with 4-0 Vicryl interrupted sutures, and the fetus was returned to the uterus. Penicillin (1 × 106 IU) was injected into the amniotic fluid, and warm saline was poured into the uterus to replace amniotic fluid lost during surgery. The hysterotomy, laparotomy, and skin incisions were closed in layers. The ewes were treated with antibiotics (oxytetracycline, 800 mg im) before the hysterotomy, and skin incisions were closed in layers. The ewes were maintained with ketamine (15 mg·kg⁻¹·h⁻¹ im). The initial ventilator settings were as follows: 60 breaths/min, 30 cmH2O peak inspiratory pressure, 4 cmH2O positive end-expiratory pressure (PEEP), inspiratory time of 0.25 s, and pause time of 0.30 s. For Sham animals, peak inspiratory pressure and respiratory rate were reduced (15–20 cmH2O and 25–35 breaths/min, respectively) to maintain normocarbia. In contrast, respiratory rate and PEEP were increased (80–90 breaths/min and 6–7 cmH2O, respectively) for DH, DH/TO, and DH/TO+R lambs in an attempt to reduce arterial PCO2 (PA CO2) and increase arterial pH. This was administered to lambs to maintain a base deficit between −2 and 2 (volume not recorded).

Parameters measured during postnatal resuscitation. Tidal volume, respiratory rate, airway pressure, arterial blood pressure and heart rate, inspiratory pressure, and PEEP were continuously recorded by a digital data logging system (PowerLab 8s, ADInstruments) connected to a computer (PowerMac 6100, Apple). Arterial blood samples (0.4 ml) were collected at 5- to 10-min intervals and analyzed for PaO2, PaCO2, and pH (I-Stat, Abbott Laboratories). After the 2-h resuscitation period, lambs were euthanized with an overdose of pentobarbital sodium administered intravenously.

Postmortem. Fetal body weight and wet lung weight were recorded. The lungs were removed, and static pressure-volume curves were performed in duplicate (31). The right lung was inflation fixed (20 cmH2O) via the trachea with 4% paraformaldehyde-2% glutaraldehyde in phosphate-buffered saline (PBS, pH 7.3). When the fixation pressure had reached a plateau, the trachea was occluded, and the lungs were submerged in buffered fixative and stored at 4°C overnight. Four portions (~3–4 g) of the left lung were oven dried at 65°C to determine pulmonary water content and dry lung weight. Sections of the left lung were also snap frozen in liquid nitrogen and stored at ~80°C.

Stereological analysis. The fixed lungs were washed for 24 h with PBS at 4°C. Right lung volume (Vl) was estimated by volume displacement of water (28). Two sections of lung tissue were obtained from the upper, middle, and lower lobes, dehydrated in alcohol, and embedded in paraffin; tissue shrinkage during processing was 17.9 ± 0.5%. Lung tissues were sectioned at 3 μm and stained with hematoxylin and eosin.

Digitized images from 45 nonoverlapping parenchymal fields were captured using a Toshiba 3CCD camera interfaced with a Leica DM2500 microscope and an IBM-PC-compatible computer. Images of lung tissue chosen for analysis contained air-exchanging parenchyma (alveoli and alveolar ducts) devoid of major airways and blood vessels. An image analysis program (SigmaScan Pro, version 5.0, SPSS) was used to calculate the percentage of parenchyma occupied by
septal tissue. Lung images were printed and examined at a final magnification of ×375. A transparent multipurpose test lattice consisting of 42 test points and a discontinuous series of line probes (Klarmann Rulings, Litchfield, NH) was placed over printed lung images and used to calculate luminal surface density (Sₜ) of the right lung. Luminal surface area of the right lung (Sₘ) was calculated according to the following equation: 

\[ S_{\text{m}} = S_{\text{t}} \cdot V_{\text{t}} \]

(2). Septal wall thickness (Tₚ) was calculated according to the following equation: 

\[ T_{\text{p}} = V_{\text{pt, tiss}} / S_{\text{m}} \]

where Vₚₜₜ is parenchymal tissue volume (6). The observer was blinded to which animal was being analyzed; observer error between duplicate counts was 2.6%.

**SP mRNA analysis.** Total cellular RNA (10 μg per fetus) was size fractioned through a denaturing agarose gel and transferred to a nylon membrane (BrightStar-plus, Ambion). Membranes were hybridized overnight (Ultrohybe, Ambion) at 42°C and probed with psoralen-biotin-labeled SP-A, SP-B, and SP-C cDNA probes at 5 pM. Ovine-specific surfactant cDNA probes were kindly provided by Dr. Fred Possmeyer (University of Western Ontario). The SP-A probe is a 277-bp Paral/KpnI cDNA (nt 1225–1504), SP-B is a 255-bp XbaI/BamHI cDNA (nt 886–1123 of the partial SP-B cDNA), and SP-C is a 145-bp AVA/XbaI cDNA (nt 71–218). Membranes were washed in saline-sodium-citrate, and the cDNA probes were detected using a streptavidin-alkaline conjugate (Ambion). SP mRNA expression and 18S rRNA levels were quantified using a computer densitometry software package (Scion Image; www.scioncorp.com).

**SP-B immunohistochemistry (AE2 cell density).** Paraffin sections obtained from the right lower lung lobe of Sham, DH, DH/TO, and DH/TO+R lambs were used for immunohistochemical staining of SP-B; rabbit anti-sheep SP-B antibody was generously provided by Dr. Jeffrey Whitsett (Cincinnati, OH). Tissues were deparaffinized, rehydrated in distilled water, immersed in antigen unmasking solution (catalog no. 3300, Vector Laboratories), and heated in a microwave oven. Sections were rinsed with distilled water and blocked with 10% normal goat serum in PBS with 0.2% Triton X-100 for 30 min at room temperature before incubation overnight (4°C) with primary antibody (1:200 in PBS). Sections were washed in PBS (0.1 M, pH 7.4) for 10 min, and endogenous peroxidase was quenched for 10 min at room temperature. Anti-sheep SP-B was detected using the Vectastain ABC goat anti-rabbit horseradish peroxidase kit (catalog no. PK6101, Vector Laboratories) and visualized using diaminobenzaldehyde substrate (catalog no. SK4100, Vector Laboratories). Tissue were lightly counterstained using Harris hematoxylin, dehydrated, and mounted using Permount (catalog no. SP15-500, Fisher Scientific). SP-B-positive cells, which are assumed to represent AE2 cells, were stained brown (see Fig. 8).

**Data analysis.** Postnatal PaO₂ and PaCO₂ were averaged over 30-min intervals and compared between groups using an unpaired Student’s t-test. For the static pressure-volume curves, lung volumes at each inflation/deflation pressure were analyzed using a two-way ANOVA, with volume and treatment as factors. Dry lung weight-to-body weight ratio, pulmonary water content, tissue-to-air space ratio, alveolar surface area, and septal wall thickness were compared between groups using an unpaired Student’s t-test. Significant difference between mean values was accepted at P < 0.05. Values are means ± SE.

**RESULTS**

**Postnatal survival and arterial blood gas status.** The survival rates and average resuscitation time of non-survivors were as follows: 11 of 11 Sham animals, 4 of 9 animals and 30 ± 8 min for the DH group, 6 of 7 animals and 60 min for the DH/TO group, and 5 of 6 animals and 40 min for the DH/TO+R group. PaO₂, PaCO₂, and pH for the four groups of lambs are presented in Fig. 1. Postnatal respiratory gas exchange was markedly impaired in DH, DH/TO, and DH/TO+R animals; these lambs were hypoxic and hypercapnic compared with Sham animals. There were no significant time-related changes in PaO₂ and PaCO₂ in DH/TO and DH/TO+R lambs during the 2-h resuscitation period. Arterial pH was not analyzed because of the influence of Tris infusion.

**Pulmonary compliance.** Restoring tracheal flow after TO did not lead to improved lung compliance (Fig. 2). Values of pulmonary compliance (adjusted for wet lung weight) in DH, DH/TO, and DH/TO+R lambs were not different from each other and were significantly lower than in Sham animals. Lung volumes measured at 40 cmH₂O and expressed in relation to wet lung weight were 160.7 ± 10.4, 29.9 ± 3.4, 42.3 ± 8.2, and 57.4 ± 30.6 ml/100 g for Sham, DH, DH/TO, and DH/TO+R animals, respectively.

**Body weight, lung weight, and pulmonary water content.** Body weight of Sham animals was significantly less (3.2 ± 0.2 kg) than that of DH (4.5 ± 0.2 kg) and DH/TO (4.3 ± 0.4 kg) lambs postmortem but was not different from that of DH/TO+R lambs (3.8 ± 0.5 kg). The rate of twin pregnancies was similar in all groups; however, four lambs from two triplet pregnancies were included in the Sham group and, thus, reduced the mean value for this group. Wet and dry lung weights were expressed, and the means ± SE are presented in Fig. 1. Postnatal survival and arterial blood gas status were as follows: 11 of 11 Sham animals, 4 of 9 animals and 30 ± 8 min for the DH group, 6 of 7 animals and 60 min for the DH/TO group, and 5 of 6 animals and 40 min for the DH/TO+R group. PaO₂, PaCO₂, and pH for the four groups of lambs are presented in Fig. 1. Postnatal respiratory gas exchange was markedly impaired in DH, DH/TO, and DH/TO+R animals; these lambs were hypoxic and hypercapnic compared with Sham animals. There were no significant time-related changes in PaO₂ and PaCO₂ in DH/TO and DH/TO+R lambs during the 2-h resuscitation period. Arterial pH was not analyzed because of the influence of Tris infusion.

**Body weight, lung weight, and pulmonary water content.** Body weight of Sham animals was significantly less (3.2 ± 0.2 kg) than that of DH (4.5 ± 0.2 kg) and DH/TO (4.3 ± 0.4 kg) lambs postmortem but was not different from that of DH/TO+R lambs (3.8 ± 0.5 kg). The rate of twin pregnancies was similar in all groups; however, four lambs from two triplet pregnancies were included in the Sham group and, thus, reduced the mean value for this group. Wet and dry lung weights were expressed, and the means ± SE are presented in Fig. 1. Postnatal survival and arterial blood gas status were as follows: 11 of 11 Sham animals, 4 of 9 animals and 30 ± 8 min for the DH group, 6 of 7 animals and 60 min for the DH/TO group, and 5 of 6 animals and 40 min for the DH/TO+R group. PaO₂, PaCO₂, and pH for the four groups of lambs are presented in Fig. 1. Postnatal respiratory gas exchange was markedly impaired in DH, DH/TO, and DH/TO+R animals; these lambs were hypoxic and hypercapnic compared with Sham animals. There were no significant time-related changes in PaO₂ and PaCO₂ in DH/TO and DH/TO+R lambs during the 2-h resuscitation period. Arterial pH was not analyzed because of the influence of Tris infusion.
were 89.3 ± 10.6 and 13.1 ± 1.2 g, respectively, for Sham, 67.5 ± 4.9 and 7.6 ± 0.5 g, respectively, for DH, 209.8 ± 21.6 and 20.7 ± 1.9 g, respectively, for DH/TO, and 100.4 ± 13.8 and 13.7 ± 1.8 g, respectively, for DH/TO+R. The dry lung weight-to-body weight ratio (Fig. 3) was reduced by 62% in DH lambs (1.6 ± 0.1 g/kg). TO increased fetal lung growth, as evidenced by normal lung-to-body weight ratios in DH/TO (5.0 ± 0.7 g/kg) and DH/TO+R (3.6 ± 0.2 g/kg) animals compared with Sham animals (4.1 ± 0.3 g/kg). Pulmonary water content (Fig. 4) was significantly higher in DH (7.8 ± 0.5 ml/g) and DH/TO (9.2 ± 0.6 ml/g) lambs than in Sham animals (5.7 ± 0.3 ml/g); values in DH/TO+R lambs (6.3 ± 0.6 ml/g) were not significantly different from those in Sham animals.

Pulmonary morphometry. Structural development of the lung was markedly impaired by DH, as indicated by increases in the tissue-to-air space ratio (0.56 ± 0.04) and septal wall thickness (6.8 ± 0.5 μm) and a reduction in alveolar surface area (5.4 ± 0.2 m²/kg body wt) compared with Sham animals (0.33 ± 0.03, 5.2 ± 0.3 μm, and 4.4 ± 0.2 m²/kg, respectively; Figs. 5...
and 6). Although periods of fetal TO increased alveolar surface area (5.4 ± 0.2 and 3.6 ± 0.4 m²/kg in DH/TO and DH/TO+R, respectively), septal wall thickness and the tissue-to-air space ratio remained higher in DH/TO lambs (6.3 ± 0.3 μm and 0.43 ± 0.02, respectively) than in Sham animals. Tissue-to-air space ratio (0.42 ± 0.06) and alveolar surface area were similar in DH/TO+R and Sham animals; however, alveolar wall thickness (7.08 ± 0.96 μm) was higher in DH/TO+R than in Sham animals.

Pulmonary SP-A, SP-B, and SP-C mRNA expression. Densitometry data obtained from Northern blots of pulmonary SP-A, SP-B, and SP-C are presented in Fig. 7. Surgical creation of DH at 65 days of gestation in fetal sheep did not significantly alter mRNA expression of SP-A, SP-B, and SP-C near term. In contrast, SP mRNA expression was markedly reduced after prolonged TO (DH/TO lambs). Restoring normal tracheal flow after a period of prolonged TO (DH/TO+R lambs) resulted in an increase in SP-A, SP-B, and SP-C mRNA expression.

SP-B immunohistochemistry. The number of epithelial cells expressing SP-B, assumed to represent AE2 cells, was markedly reduced after prolonged TO (DH/TO; Fig. 8). In contrast, the number of AE2 cells was increased in animals that underwent temporary TO (DH/TO+R). The density of SP-B-positive cells did not appear to be different between the DH and Sham animals.

DISCUSSION

We have shown that temporary TO in fetal sheep with severe lung hypoplasia increases lung tissue growth and restores SP mRNA and SP-B expression but does not lead to normal postnatal lung function. The lungs from DH/TO+R lambs were stiff and were unable to effectively function in respiratory gas exchange. Temporary TO did not improve postnatal respiratory function above that in animals that underwent prolonged TO. The increase in septal wall thickness observed in untreated DH animals was not corrected by temporary or prolonged TO. We believe that increased septal wall thickness contributed to reduced lung compliance and impaired gas exchange in animals that underwent temporary TO for treatment of severe lung hypoplasia.

Prenatal treatment of lung hypoplasia by prolonged TO can restore respiratory function, provided exogenous surfactant is administered at birth (25). In lambs with DH created at 78 days of gestation that underwent TO between 110 and 141 days of gestation, O’Toole and colleagues (25) observed normal levels of arterial oxygenation and pulmonary blood flow in the immediate (i.e., 4 h) postnatal period. It is important to note that the intratracheal administration of surfactant to lambs that underwent prolonged TO was crucial in establishing normal pulmonary function and that respiratory gas exchange and pulmonary blood flow were markedly impaired in animals that were not given surfactant (25). The DH/TO lambs in our study were not treated with surfactant; they were markedly hypoxic and hypercapnic after birth and exhibited low lung compliance. It is likely that the reduction in pulmonary surfactant gene expression and loss of alveolar epithelial SP-B-positive cells, combined with increased

Fig. 6. Representative photomicrographs from Sham (A), DH (B), DH/TO (C), and DH/TO+R (D) fetuses. Right lung was inflation fixed via the trachea at 20 cmH₂O with 4% paraformaldehyde-0.2% glutaraldehyde. Percentage of lung occupied by parenchymal tissue and interalveolar wall thickness are markedly increased in DH lambs compared with Sham animals. Fetal TO reduced percentage of lung occupied by tissue; however, alveolar wall thickness remained increased (P < 0.05) in DH/TO and DH/TO+R lambs.
septal wall thickness, were the primary causes of poor postnatal outcome in DH/TO lambs.

It is now evident that the degree to which the lung is expanded with fluid during fetal life has profound effects on the differentiation of alveolar epithelial cells. Increased fetal lung tissue stretch induced by TO accelerates the normal differentiation of AE2 cells into AE1 cells, resulting in reduced AE2 cell density and SP mRNA expression (3, 4, 13, 14, 26). We have shown in fetal sheep with severe lung hypoplasia that 4 wk of TO is sufficient to induce an almost total loss of SP-B-positive alveolar epithelial cells (i.e., the AE2 cells). Although there is poor understanding of the factors that regulate the transition of AE2 into AE1 cells, there is evidence that mechanical stimuli play an important role in this process. Isolated AE2 cells cultured on “attached” collagen gels develop characteristics of AE1 cells and lose their ability to express SP mRNA (8, 30). However, when gels were detached from the culture dish, the change in cell shape caused contraction of the floating gels and was associated with a change in cell phenotype from AE1 to AE2 cells, as evidenced by the reacquisition of lamellar bodies and apical microvilli and reexpression of SP mRNA (8, 30). Sustained mechanical stretch of cultured rat AE2 cells also increases the number of cells expressing the AE1 cellspecific marker rTL40 and decreases SP-B and SP-C mRNA expression (15). Morphological studies in fetal sheep have demonstrated that prolonged periods of increased fetal lung expansion induced by TO decreases AE2 cell number and increases AE1 cell number (4, 13, 14). Taken together, these studies provide evidence that mechanical signals regulate the transition of AE2 to AE1 cells.

Stretch-induced differentiation of AE2 into AE1 cells during TO may be reversed if the levels of lung tissue stretch are returned to normal. That is, AE1 cells are not terminally differentiated during periods of increased fetal lung tissue stretch. In sheep, the number of AE2 cells increases when normal tracheal fluid flow is restored after a period of prolonged TO (5). Recently, Flecknoe et al. (14) measured changes in alveolar epithelial cell phenotype in fetal sheep that had undergone a period of increased lung expansion (induced by TO) followed by a period of lung underexpansion (induced by lung liquid drainage). They showed that a sustained period of fetal lung underexpansion after 10 days of TO promoted an increase in AE2 cell number and a decrease in AE1 cell number (14). It is believed that continuous regulatory input is required to maintain the differentiation state of alveolar epithelial cells (8) and that AE1 cells can transdifferentiate into functional (i.e., surfactant-producing) AE2 cells if the levels of lung tissue stretch are reduced after prolonged TO. We have shown that SP gene expression and AE2 cell density were increased in lambs that experienced 10 days of normal tracheal flow after 3 wk of TO (i.e., DH/TO+R animals), which is consistent with findings from previous studies.

An intriguing finding of our study was that the lungs of DH/TO+R lambs were abnormally stiff, despite their higher levels of SP mRNA expression and AE2 cell density, than the lungs of DH/TO animals. There are several plausible explanations for the abnormal postnatal gas exchange and lung tissue compliance in DH/TO+R lambs. First, there is evidence to suggest that production of surfactant phospholipids by AE2 cells may be reduced in lambs after temporary TO. Bratu and colleagues (7) recently reported a marked decrease in phosphatidylcholine content of bronchoalveolar lavages from neonatal lambs that underwent temporary prenatal TO to reverse an existing lung hypoplasia. Phosphatidylcholine is the major lipid component of alveolar surfactant that is synthesized exclusively by AE2 cells and is essential for reducing alveolar surface tension after birth (18). Further studies are needed to determine whether AE2 cell function, particularly the ability to produce surfactant phospholipids, is altered when normal tracheal flow is restored after periods of prolonged TO.
A persistent increase in septal wall thickness observed in lambs that underwent temporary (DH/TO+R) or prolonged (DH/TO) TO may also reduce lung tissue compliance and limit postnatal gas exchange. We were surprised that septal wall thickness in DH lambs was not attenuated by prolonged TO, because 4 wk of TO has previously been shown to result in “thinner future alveolar walls” in normally grown fetal sheep (1). We speculate that longer periods of TO (i.e., >4 wk) may be required to reduce septal wall thickness in fetuses with severe lung growth disorders. Indeed, in fetal sheep with DH-induced lung hypoplasia, thinner septal walls have been observed after 6 wk of TO, but not after 2 wk of TO (22). In our present study, the duration of TO could not be maintained for >4 wk because of the risk of spontaneous delivery of lambs at full term (~145 days). Although TO could have been performed earlier in gestation to achieve a longer period of TO, the severity of lung hypoplasia at the time of TO would have been less. Because clinical trials of TO are reserved for severe and/or lethal forms of lung hypoplasia, we designed our study so that we could examine the effects of TO in fetuses with severe lung growth deficits. To achieve this, we chose to maximize the time between surgical creation of DH (65 days) and TO (110 days) before commencing 4 wk of TO.

It has been proposed that, during lung development, thinning of septal walls occurs via a change in the balance between the rate of proliferating and apoptotic cells (29). In newborn rats, attenuation of alveolar walls during the 3rd wk of life is associated with an increased rate of programmed cell death of fibroblasts and AE2 cells (29). Attenuation of alveolar walls in sheep occurs predominantly during late fetal life and is regulated, in part, by the levels of endogenous glucocorticoids (i.e., cortisol) (29); it is unknown whether increased apoptosis contributes to alveolar wall thinning in fetal sheep. In contrast, cell proliferation is increased after TO and results in a transient increase in distance between air spaces (i.e., thicker air space walls) (24, 27). In midgestation fetal sheep, TO stimulates proliferation of mesenchymal cells (27), whereas increased division of fibroblast and type II cells occurs during TO in late-gestation sheep (24). Because lung development is delayed in animals with severe lung hypoplasia, it is conceivable that late-gestation TO in fetuses with DH may increase septal wall thickness via preferential division of mesenchymal cells. Further experiments are required to test this hypothesis. Prenatal administration of glucocorticoids has been shown to enhance alveolar wall thinning in fetuses with lung hypoplasia (19). It is possible that treatment with a combination of TO and glucocorticoids may result in a more “structurally normal” lung that can function effectively at respiratory gas exchange after birth.

Fig. 8. SP-B immunostaining of lung tissues obtained from Sham (A), DH/TO (B), and DH/TO+R (C) lambs at ~139 days of gestation. Alveolar epithelial cells expressing SP-B, assumed to represent AE2 cells, were abundant in Sham and DH/TO+R animals (located at tip of arrowhead), whereas AE2 cell density was decreased after prolonged TO (DH/TO). Sections were counterstained with Harris hematoxylin. Scale bar, 50 μm.
Abnormal pulmonary vascular growth in lambs that underwent TO may contribute to impaired postnatal gas exchange. The pulmonary vasculature was not examined in this study; however, other groups have shown that the innervation of alveolar walls with capillaries appears to be normal after periods of stretch-induced lung growth (10, 22). There is little knowledge of the effects of prolonged TO on growth of larger-diameter pulmonary vessels. Moreover, it is unknown whether stretch-induced fetal lung growth alters mechanisms responsible for increasing pulmonary blood flow in the immediate newborn period (e.g., synthesis of vasoreactive substances, such as nitric oxide and endothelins).

An interesting and unexpected finding of our study was that pulmonary water content was ~60% higher in DH/TO than in Sham animals. We speculate that the increased pulmonary water content in DH/TO lambs may be secondary to reduced alveolar SP content. Pulmonary surfactant reduces alveolar surface tension, thereby altering alveolar SP content at 2 h after birth. In treating fetuses with severe lung hypoplasia by TO, we suggest that it may be necessary to achieve normal lung tissue structure (e.g., thinner septal walls) to reduce lung compliance and enhance respiratory gas exchange after birth.

The authors thank Antoneta Radu for preparing lung tissues for stereological analysis, Dr. Miguel Esteves for assistance with Northern blot analysis, and Lee Barthel for technical assistance during surgery preparations.

REFERENCES


