RESEARCH ARTICLE

Effects of antenatal melatonin therapy on lung structure in growth-restricted newborn lambs

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1The Ritchie Centre, Hudson Institute of Medical Research, Clayton, Victoria, Australia; 2Department of Obstetrics and Gynaecology, Monash University, Clayton, Victoria, Australia; 3Monash Newborn, Monash Medical Centre, Melbourne, Victoria, Australia; 4Monash Micro Imaging, Monash University Clayton, Victoria, Australia; and 5Department of Anatomy, Biochemistry, and Molecular Biology, Monash University, Clayton, Victoria, Australia

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Polglase GR, Barbuto J, Allison RJ, Yawno T, Sutherland AE, Malhotra A, Schulze KE, Wallace EM, Jenkin G, Ricardo SD, Miller SL. Effects of antenatal melatonin therapy on lung structure in growth-restricted newborn lambs. J Appl Physiol 123: 1195–1203, 2017. First published August 17, 2017; doi:10.1152/japplphysiol.00783.2016.—Oxidative stress arising from suboptimal placental function contributes to a multitude of pathologies in infants compromised by fetal growth restriction (FGR). FGR infants are at high risk for respiratory dysfunction after birth and poor long-term lung function. Our objective was to investigate the contribution of oxidative stress to adverse lung development and the effects of melatonin administration, a powerful antioxidant, on lung structure in FGR lambs. Placental insufficiency and FGR was surgically induced in 13 fetal sheep at ~105 days of gestation by ligation of a single umbilical artery. Maternal intravenous melatonin infusion was commenced in seven of the ewes 4 h after surgery and continued until birth. Lambs delivered normally at term and lungs were collected 24 h after birth for histological assessment of lung structure and injury and compared with appropriately grown control lambs (n = 8). FGR fetuses were hypoxic and had lower glucose during gestation compared with controls. Melatonin administration prevented chronic hypoxia. Within the lung, FGR caused reduced secondary septal crest density and altered elastin deposition compared with controls. Melatonin administration had no effect on the changes to lung structure induced by FGR. We conclude that chronic FGR disrupts septation of the developing alveoli, which is not altered by melatonin administration. These findings suggest that oxidative stress is not the mechanism driving altered lung structure in FGR neonates. Melatonin administration did not prevent disrupted airway development but also had no apparent adverse effects on fetal lung development.

NEW & NOTEWORTHY Fetal growth restriction (FGR) results in poor respiratory outcomes, which may be caused by oxidation in utero. We investigated the contribution of oxidative stress to adverse lung development and the effects of melatonin administration, a powerful antioxidant, on lung structure in FGR lambs. FGR disrupted septation of the developing alveoli, which is not altered by melatonin administration. Oxidative stress may not be the mechanism driving altered lung structure in FGR neonates.

fetal growth restriction, intrauterine growth restriction; melatonin; small for gestational age; antioxidant

FETAL GROWTH RESTRICTION (FGR) is one of the most common and serious complications of pregnancy, in which suboptimal fetal growth results in a fetus that is smaller than it should be for a given gestational age. FGR affects up to 9% of pregnancies in high-resource countries (25). FGR infants have an increased incidence of perinatal mortality and morbidity than appropriately grown infants whether they are born preterm or at term (35), with a 10- to 20-fold greater risk of perinatal mortality compared with appropriately grown infants (40). After birth, growth-restricted infants also have significantly increased risk of neonatal complications, including respiratory distress and the need for respiratory support, chronic lung disease, abnormal glucose regulation, difficulties in maintaining blood pressure, necrotising enterocolitis, low Apgar scores, and neurodevelopmental disorders and functional delay (34, 37, 40, 50).

Placental insufficiency, resulting in chronic fetal hypoxia, is the leading cause of FGR. The FGR fetus adapts to its sustained low-oxygen environment by altering cardiovascular output to favor blood flow to the brain, heart, and adrenal glands to optimize oxygen and nutrient supply to these vital organs (23). These cardiovascular adaptations result in distinctive asymmetric growth restriction, characterized as sparing of head and brain growth at the expense of other organ development, including the lungs. Accordingly, FGR infants are at significantly increased risk of pulmonary hypertension and development of the chronic lung disease bronchopulmonary dysplasia (BPD) (6, 8). Experimentally, ovine FGR induced by short-term placental insufficiency at midgestation does not induce gross structural changes in the fetal lung (3, 46), but longer-term FGR conducted later in gestation is associated with increased thickness of the alveolar blood-air barrier in lambs that persists into adulthood (22).

In addition to chronic hypoxia, it is hypothesized that fetal pathologies evident in FGR are mediated by oxidative stress arising from poor placental function (13, 27, 33). Melatonin has recently received interest in perinatal research because of its strong antioxidant properties that likely contribute to its neuroprotective role in central nervous system development (27, 31) and prevention of cardiovascular dysfunction during hypoxic development (16). The administration of melatonin to pregnant sheep has shown that it crosses the placenta (29) and...
reduces circulating and brain markers of oxidative stress in response to acute and chronic fetoplacental hypoxia (31, 51, 53). Human clinical trials are now underway to assess whether antenatal maternal melatonin administration reduces fetoplacental oxidative stress and improves outcome in pregnancies complicated by FGR and/or preeclampsia (2, 15). Oxidative stress is also associated with injury to immature lungs (54) and melatonin administration to preterm newborns with acute respiratory distress improves lung outcomes (11). Accordingly, in this study we set out to examine lung pathology associated with chronic placental insufficiency and FGR using our established ovine model and to determine whether chronic antenatal maternal melatonin administration improves lung structure in FGR newborn lambs.

METHODS

Ethics

The surgical and experimental procedures undertaken in this project were approved by the Monash Medical Centre Animal Ethics Committee (approval no. MMCA2010/03).

Animal Surgery

The animal procedures for this study have been described in detail previously (31). Of the n = 30 lambs presented in that study, we collected and analyzed lungs from n = 21 lambs, which are described herein for this study.

Briefly, aseptic surgery was performed on 21 anesthetized Border-Leicester pregnant ewes carrying a single fetus at 105–110 days gestational age. Prior to the induction of anesthesia, all ewes received 1 g of ampicillin (Austrapen; Lennon Healthcare, St. Leonards, NSW, Australia) and 500 mg of engemycin (Coopers, Bendigo East, VIC, Australia) intravenously (iv). The fetus was exposed via caesarean section, and a sterile polyvinyl catheter (inner diameter 0.8 mm, outer diameter 1.5 mm; Critchley Electrical, Kingsgrove, NSW, Australia) containing 0.9% saline and heparin (25,000 IU/l; Pfizer Australia, West Ryde, NSW, Australia) was placed into the femoral artery. Single umbilical arterial ligation was performed on 13 fetuses to induce fetal growth restriction (FGR) by placing two silk ligatures tightly around one of the umbilical arteries, and the umbilical cord sheath was then repaired. In eight control fetuses, the umbilical cord was handled, but the artery was not ligated. The fetus was returned to the uterus, and the uterine and abdominal incisions were repaired. A maternal jugular vein catheter was inserted for antibiotic and melatonin administration before the ewe was recovered.

Experimental Design

Melatonin was administered to seven of the 13 ewes carrying a FGR fetus (FGR + MLT). Melatonin (Sigma-Aldrich, Castle Hill, NSW, Australia) was dissolved in absolute ethanol and diluted to 0.08 mg/ml using sterile 0.9% saline, so that the concentration of ethanol in the final solution was 1%. Melatonin treatment began 4 h after surgery with a 1-mg melatonin bolus in 5 ml of saline (iv), and ewes were then placed on a continuous melatonin infusion of 0.25 mg·3.16 ml·1·h⁻¹, thereby delivering 6 mg melatonin every 24 h (equivalent to 0.1 mg/kg iv); this is well below the melatonin dosage of >20 mg/kg considered to be anesthetic (18). The continuous infusion was delivered for the remainder of the pregnancy with a CADD infusion pump (Smiths Medical, St. Paul, MN), which was refilled daily. Fetal arterial catheters were flushed with 5 ml of heparinized (50 IU/ml) saline every 2nd day to maintain patency.

Fetal arterial blood samples were collected at gestational age 110, 115, 120, 125, 130, 135, 140, and 145 days for assessment of pH, oxygen saturation (%SaO₂), partial pressure of oxygen (Pao₂), partial pressure of carbon dioxide (Paco₂), lactate, and glucose (ABL700; Radiometer, Copenhagen, Denmark). Ewes were allowed to deliver normally at ~147 days gestation, with an investigator present at the time of birth to cut the fetal arterial catheter. Lambs were kept with their mothers; if they were not able to self-feed from their mother they were bottle-fed lamb formula (Sheep Milk Replacer; Wombaroo Food Products) at 4-h intervals until 24 h of age. At 24 h after birth, lambs were euthanized by iv injection of pentobarbitone sodium (Lethabarb; Virbac, Peakhurst, NSW, Australia).

Histological Analysis

The right upper lung lobe was inflation fixed (at 20 cmH₂O) with 10% formalin, and samples were embedded in paraffin. Three blocks were randomly selected from different regions of the right upper lobe (upper, middle, and lower), and 5-μm sections were stained with hematoxylin and eosin (H & E) and used to score lung injury. A total of 15 random high-power fields were scored on a 0–4 scale (0 = none, 4 = severe pathology) for septal thickness (0 = thin distal airway walls, 2 = mild thickening of airway walls with thin alveolar regions, and 4 = thickening of all airspaces), hemorrhage (0 = no hemorrhage, 2 = hemorrhage into parenchyma and small airway hemorrhages, and 4 = large airway hemorrhage), inflammation (0 = no inflammatory cells, 2 = 1–5 cells/hpf, and 4 = >5 inflammatory cells/hpf), and epithelial sloughing (0 = no sloughing, 2 = epithelial wall disruption, and 4 = denuded epithelium in airspace) (14). A total injury score was determined by the addition of each of the four parameters.

Collagen. Lung sections (5 μm) were stained with picrosirius red. The birefringence of the collagen was visualized using a Leica Abrio polarizing microscope (512 × 512 CCD black and white camera connected to a Leica Abrio software). Five images per section were obtained, using a 40× objective lens. Analysis was performed using the image processing package Fiji. The total area of collagen within each image was calculated and normalized to the area of tissue.

Elastin. Lung elastin content was determined on sections stained with Hart’s for elastin fibers. Visualization of elastin fibers was performed using Olympus Provis AX70 light microscope with a color digital camera (Olympus DP70) and SI AnaySis software. Five images per section were obtained, and a grid overlay with 1200 dots was placed over the image, capturing using a 20× objective lens. The number of dots landing on tissue, airspace and secondary septal crests were counted. The number of secondary septal crests was divided by the area of tissue and multiplied by 100 to yield a secondary septal crest percentage.

Immunohistochemical Analysis

Immunohistochemistry for 8-hydroxy-2'-deoxyguanosine (8-OHdG), a marker of DNA damage induced via oxidative stress sheep serum, was performed on 5-μm paraffin-embedded lung tissue sections.

In brief, sections were dewaxed and rehydrated in serial alcohols. Antigen retrieval was performed in citrate buffer and blocked with 10% BSA in 0.1% Tween-20 PBS. The primary 8-OHdG antibody was applied to 5-μm lung sections (1:200, JaIC; NIKKEN SEIL). All sections were treated with a secondary antibody (1:200; biotinylated α-mouse IgG antibody; Vector Laboratories, Burlingame, CA). Staining was revealed using 3,3-diaminobenzidine (Pierce Biotechnology, Rockford, IL), and sections were counterstained with Harris hematoxylin, dehydrated, mounted, and viewed using light microscopy for assessment.

Slides were scanned (Aperio Scanscope AT Turbo) and images extracted using Aperio Imagescope software. Five images per section were obtained. Strong nuclear staining indicated the presence of antigen. Only a strong specific immunohistochemical reaction was considered as positive staining. The total number of positive cells within each image was counted and normalized to the area of total cells.
Table 1. Primer sequences for quantitative real-time PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Species</th>
<th>Accession No.</th>
<th>Primer Sequence</th>
<th>Amplicon Length, nt</th>
</tr>
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<tr>
<td>18S</td>
<td>Rat</td>
<td>X01117</td>
<td>5'-GTAAACCGTTGAACCCCAT-3', 5'-CCATCCAATCGTATAGGG-3'</td>
<td>105</td>
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<td>Elastin</td>
<td>Ovine</td>
<td>M26189</td>
<td>5'-ATCTCTGAGTCTAGCAGTATGAG-3', 5'-GTTTGGTGGGAAAGAAGCA-3'</td>
<td>66</td>
</tr>
<tr>
<td>Collagen III</td>
<td>Ovine</td>
<td>DQ239680</td>
<td>5'-CTGGGAAAATGCGTGAAG-3', 5'-GTCAACAGAGGGCGGAT-3'</td>
<td>65</td>
</tr>
<tr>
<td>Collagen IV</td>
<td>Ovine</td>
<td>L47282</td>
<td>5'-GGAGATAACGAGCCAATAC-3', 5'-GAAGGCCAGGAAACTGTA-3'</td>
<td>90</td>
</tr>
</tbody>
</table>

Total lung protein concentration. Total protein concentration was determined from frozen samples of lung tissue using the Pierce BCA Protein Assay Kit (Thermo Scientific, Life Technologies).

Malondialdehyde concentration. Malondialdehyde concentration, a measure of lipid peroxidation, was assessed from fetal plasma samples and in frozen lung tissue obtained at postmortem, using the thiobarbituric acid reactive substances (TBARS) method (Cayman Chemical and in frozen lung tissue obtained at postmortem, using the thiobarbituric acid reactive substances (TBARS) method (Cayman Chemical TBARS Assay Kit no. 10009055; Sapphire Bioscience).

Real-time PCR. Fetal sheep lung tissue was homogenized and total RNA isolated (RNasey Midi Kit; Qiagen) and reverse-transcribed into cDNA (SuperScript III reverse transcriptase; Invitrogen). Genes of interest were measured by quantitative RT-PCR using Applied Biosystems 7900HT Fast Real-Time PCR system. Relative mRNA expression of elastin, collagen III, and collagen IV (see Table 1 for details) was measured. The expression of all genes was normalized to the 18S rRNA for each sample using the cycle threshold (ΔCt) method of analysis and was expressed relative to the control group.

Statistical Analysis

Serial fetal blood gas variables were compared using two-way repeated measures ANOVA (SigmaPlot version 12.0; Systat Software). Holm-Sidak multiple-comparisons post hoc test was used to determine differences between groups. Histological analyses were compared using one-way ANOVA. Statistical significance was accepted for P < 0.05. Data are presented as means ± SE.

RESULTS

Fetal Characteristics

During pregnancy, FGR fetuses had a significant reduction in fetal arterial PaO2, PaCO2, and SaO2 at 125 and 130 days gestation (Fig. 1) compared with control lambs. FGR lambs remained hypoxic, but differences between groups were not significant thereafter. PaO2, PaCO2, and SaO2 were not different in FGR + MLT fetuses compared with control at any gestational age. Fetal circulating glucose levels were lower in FGR lambs compared with control and FGR + MLT lambs from 120 to 135 days gestation (Fig. 1). Fetal lactate levels were not different between the groups over the duration of fetal monitoring (data not shown).

We measured fetal plasma malondialdehyde levels in all animals where a full cohort of blood samples was available at each gestational age (110–145 days); n = 3 control, n = 4 FGR, and n = 5 FGR + MLT. Malondialdehyde is a late-stage marker of lipid peroxidation and oxidative stress. Fetal plasma malondialdehyde levels were higher in FGR fetuses compared with control and FGR + MLT fetuses (Fig. 1F), but overall the difference was not significant (P = 0.13), which was likely due to low numbers of animals per group.

![Graphs](https://via.placeholder.com/150)

Fig. 1. Partial pressure of arterial oxygen (PaO2; A), partial pressure of arterial carbon dioxide (PaCO2; B), pH (C), arterial hemoglobin saturation with oxygen (D), arterial glucose concentration (E), and plasma malondialdehyde (MDA; F) concentration in control, fetal growth restriction (FGR), and FGR lambs that received melatonin (FGR + MLT), measured after surgical intervention. Note that for the malondialdehyde date, data were available only for n = 3 (control), n = 4 (FGR), and n = 5 (FGR + MLT). Data presented as means ± SE *Significant difference, FGR vs. control.

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Lamb birth characteristics are presented in Table 2. Single umbilical artery ligation resulted in a 25% reduction in body weight at birth, persisting until 24 h. Melatonin infusion did not alter this effect. Brain weight was not different between groups; however, brain/body weight ratio was significantly increased in FGR and FGR + MLT lambs compared with controls, indicative of asymmetric growth restriction. Lung weight was significantly reduced in FGR and FGR + MLT lambs compared with controls, but this difference was lost when adjusted for body weight.

Assessment of Lung Structure and Composition

Elastin. Figure 3 shows representative elastin airway distribution for all three groups of lambs. The percentage of lung occupied by tissue or airspace was not different between groups. Secondary septal crest density was significantly reduced in FGR and FGR + MLT lambs compared with control lambs. Elastin deposition was altered in the alveolar walls of lungs from FGR and FGR + MLT lambs compared with controls. Qualitatively, elastic fibers were more likely to be located along the saccule wall rather than being focused at the

Table 2. Lamb characteristics

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control (n = 8)</th>
<th>FGR (n = 6)</th>
<th>FGR + MLT (n = 7)</th>
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<tbody>
<tr>
<td>Males/females</td>
<td>4/4</td>
<td>5/1</td>
<td>2/5</td>
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<tr>
<td>GA at delivery, days</td>
<td>145 ± 1.8</td>
<td>145 ± 0.8</td>
<td>145 ± 1.0</td>
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<tr>
<td>Body weight at birth, kg</td>
<td>4.51 ± 0.24</td>
<td>3.08 ± 0.47*</td>
<td>3.17 ± 0.20*</td>
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<tr>
<td>Body weight at 24 h, kg</td>
<td>4.74 ± 0.20</td>
<td>3.28 ± 0.56</td>
<td>3.33 ± 0.23</td>
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<tr>
<td>Brain weight, g</td>
<td>56.04 ± 1.70</td>
<td>52.03 ± 2.18</td>
<td>51.32 ± 1.88</td>
</tr>
<tr>
<td>Brain/body weight, g/kg</td>
<td>11.90 ± 0.36</td>
<td>17.64 ± 2.1*</td>
<td>15.68 ± 1.04*</td>
</tr>
<tr>
<td>Lung weight, g</td>
<td>109.67 ± 6.86</td>
<td>75.52 ± 12.17*</td>
<td>82.51 ± 12.7*</td>
</tr>
<tr>
<td>Lung/body weight, g/kg</td>
<td>23.29 ± 1.46</td>
<td>23.32 ± 1.65</td>
<td>24.38 ± 2.78</td>
</tr>
</tbody>
</table>

Values are means ± SE. FGR, fetal growth restriction; FGR + MLT, FGR lambs that received melatonin; GA, gestational age. *P < 0.05 compared with control.

Fig. 2. A–F: representative lung sections stained with Hart’s to view elastin content (black staining) at ×40 magnification in control (A), FGR (B), and FGR + MLT lambs (C) and ×100 magnification in control (D), FGR (E), and FGR + MLT (F). Arrows indicate elastin deposition at the points of septa in control lambs, which is disordered in FGR and FGR + MLT lambs. G: density of secondary septal crests expressed as %total tissue content determined by point counting. H and I: %tissue (hatched bars) and airspace (solid bars) (H) and relative expression of elastin mRNA (I). *Significant difference from control.
tips of secondary septa. This is indicative that alveolarization was disorganized, as many focal sites of elastin aggregation were not associated with the formation of secondary septal crests (Fig. 2). Expression of elastin mRNA was higher in the FGR groups compared with controls.

**Collagen.** Figure 3 shows representative collagen airway distribution for the three groups studied. Qualitative assessment of collagen distribution found no gross differences in the distribution of collagen within the parenchyma or alterations in the orientation of the fibers (Fig. 3). Quantitative assessment of total tissue collagen content found no differences between groups (Fig. 3). Furthermore, expression of collagen III and collagen IV mRNA was not different between groups.

**Assessment of Lung Injury**

Wall thickness, inflammatory cell infiltration, hemorrhage, epithelial sloughing, and total injury score, which was assessed on H & E-stained lung sections, were not different between groups (Fig. 4).

Total lung tissue protein concentration was not different between groups (control: 7.567 ± 0.18 μg/ml; FGR: 7.375 ± 0.299 μg/ml; FGR + MLT: 7.839 ± 0.419 μg/ml). Similarly, malondialdehyde concentration in lung tissue measured 24 h after birth was not different between groups (control: 9.3 ± 1.1 μM; FGR: 8.1 ± 1.3 μM; FGR + MLT: 9.5 ± 1.5 μM).

**Oxidative Stress Marker 8-OHdG**

Oxidative stress marker 8-hydroxy-2′-deoxyguanosine (8-OHdG) was assessed by immunohistochemistry (Fig. 5). 8-OHdG was significantly reduced in FGR lambs (control vs. FGR: 2.8 ± 0.6 vs. 1.0 ± 0.4%), whereas treatment with melatonin had no effect on 8-OHdG (FGR + MLT: 1.2 ± 0.3%).

**DISCUSSION**

The contribution of oxidative stress to adverse lung development in FGR infants and the effects of melatonin administration on lung structure are not known. This is important, since clinical trials are now underway to treat pregnancies complicated by FGR and/or preeclampsia with antenatal melatonin (2, 15). Using our established model of placental insufficiency and subsequent fetal growth restriction, we assessed the effects of FGR with or without antenatal melatonin exposure on lung structure in newborn lambs. Our results show that chronic placental insufficiency induced via single umbilical artery ligation results in FGR and altered lung development, with reduced secondary septal crest density and dysmorphic elastin distribution within the airways. Exposure to chronic antenatal melatonin was benign to the FGR lung, with no difference in lung morphology in FGR lambs with and without antenatal melatonin treatment.

Chronic placental insufficiency, experimentally induced by single umbilical artery ligation, induced chronic fetal hypoxia,
hypoglycemia, and asymmetric fetal growth restriction, consistent with the known pathophysiology in human FGR (43) and with our previous experimental findings where we also described increased lactate production (24, 26, 45, 46). In this study, placental insufficiency was initiated at 0.7 gestation, representing late-onset FGR, during the late canalicular stage of lung development. Late-onset FGR is the most common form of growth restriction, present in ~80% of FGR infants (9). Our results demonstrate that chronic fetal hypoxia and hypoglycemia do not alter lung weight, which was normal in FGR lambs when adjusted for body weight. However, assessment of lung architecture demonstrated a significant alteration in lung development, with reduced secondary septal crest density in FGR lambs. The reduction of secondary septal crests is indicative of an inhibition of alveolarisation in FGR lambs. Furthermore, in FGR lambs, elastin was laid down in a disordered manner in the alveolar walls, which was most evident at focused points where secondary septal crests would be expected to form. This observation is consistent with the suggestion that septation is inhibited following FGR (21, 22). Impaired alveolarisation is also observed in other ovine models of growth restriction induced using hyperthermia (41), undernutrition (17), or placental embolization (21, 22). The reduction in secondary septa likely occurs in response to chronic hypoxia, which may slow down or prevent alveolar formation, particularly inhibiting the critical and rapid increase that normally occurs after 140 days gestation in fetal sheep (10, 21). Interestingly, the reduction in secondary septa previously demonstrated in fetal and postnatal lambs (21, 22) showed a concurrent increase in extracellular matrix thickening. In the current study, we observed a small but nonsignificant increase in wall thickening in FGR lambs; however, we did not observe changes to the percentage of lung tissue or airspace.

We observed reduced septation in response to 40 days of placental insufficiency, which is in contrast to our previous

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<tr>
<th>Control</th>
<th>FGR</th>
<th>FGR + MLT</th>
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<td>A</td>
<td>B</td>
<td>C</td>
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Fig. 4. Representative hematoxylin and eosin-stained lung sections in control, FGR, and FGR + MLT lambs (A–C). Assessment of lung inflammation parameter wall thickness (0–4; D), inflammation (0–4; E), hemorrhage (0–4; F), epithelial sloughing (0–4; G), total injury score (0–16; H), and total lung protein concentration (I). Data are presented as means ± SE.
finding in this model using a much shorter duration of FGR (46). In our previous study, lungs were inspected 7 days after the onset of placental insufficiency and FGR (46), and thus, long-term exposure to chronic hypoxia and hypoglycemia adversely affects lung development, whereas a short-term exposure did not. Furthermore, the reduction in septation is more likely to be apparent later in gestation when there is a rapid increase in alveolar number (10, 21). Combined, these findings demonstrate that both the timing and duration of placental insufficiency affects lung structure. Indeed, the simplification of the alveoli observed in this study may underlie the increased risk of BPD in FGR neonates observed clinically (4, 12, 36, 44).

In addition to chronic hypoxia, it is hypothesized that increased oxidative stress, arising from placental pathology, contributes to suboptimal organ development and cardiovascular deficits in FGR infants (13, 16, 27, 33). Melatonin demonstrates powerful antioxidant properties, can be administered to the mother and reaches the fetus, is safe, and has been shown to reduce circulating and brain markers of oxidative stress in response to acute and chronic fetoplacental hypoxia (31, 51, 53). Given that oxidative stress is associated with injury to the immature lungs (54), we investigated whether melatonin could reduce oxidative stress within the lung and improve structural lung deficits associated with FGR. Circulating malondialdehyde concentration was elevated in FGR fetuses between 115 and 130 days gestation, albeit nonsignificantly compared with control and FGR + MLT lambs. Malondialdehyde is raised in response to end-stage lipid peroxidation, and therefore, it is considered a marker of pronounced oxidative stress. In our model we are unable to determine whether malondialdehyde concentration is increased due to increased production or whether degradation is decreased. Chronic antenatal hypoxia in guinea pigs induces an ~20% increase in fetal lung tissue malondialdehyde (1), but at 24 h after birth, we did not find a difference in lung tissue malondialdehyde concentration or in oxidative stress marker 8-OHdG. This is not surprising given that all lambs were 24 h old at tissue collection and had been in air-breathing, normoxic conditions since birth. Certainly, the results of the current study do not support that oxidative stress is a principal contributor toward altered lung development in FGR infants given that lung structural simplification was similarly affected in FGR and FGR + MLT lambs, but neither circulating or lung oxidative stress was elevated in melatonin-treated animals.

A further contention is whether melatonin levels reached therapeutic levels within the lung, as they do in the brain. During fetal life, the lung receives only 10% of right ventricular output due to the presence of lung liquid, with the majority of blood flow bypassing the lungs through the ductus arteriosus (42). Thus it is highly likely that the melatonin passing into the fetal circulation will have the majority of its effect systemically rather than pulmonary.

A notable beneficial effect of melatonin in the current study was improved fetal oxygenation and circulating glucose concentration. Although it is important to note that we are unable to...
to determine whether this results from improved delivery or extraction or from a reduction in consumption, FGR fetuses alone demonstrated chronic hypoxemia and hypoglycemia, characteristic responses to placental insufficiency, and FGR + MLT fetuses did not. This finding supports that maternal melatonin administration improves placental function. Previous work demonstrates melatonin’s ability to increase placental efficiency and birth weight in undernourished rats (38) and to increase umbilical blood flow in fetal sheep (20, 48). These utero-placental benefits are likely to be mediated by the actions of melatonin to increase placental antioxidant production (38) and to increase nitric oxide bioavailability (48). However, improved fetal oxygenation and glucose supply did not ensure normal lung development in FGR + MLT lambs. Thus, altered lung development in growth-restricted fetuses appears to be driven by other pathways. Indeed, we have reported previously that ovine placental insufficiency and FGR is associated with a fetoplacental inflammatory response with elevated proinflammatory cytokines and prostaglandin E₂ (5). Given that intrauterine inflammation has profound influences on lung development (19) with only modest activation of oxidative stress pathways (7), it is likely that inflammation is the primary cause of suboptimal lung development in our study. More studies are required to elucidate the mechanisms in which FGR alters fetal lung development.

The current study is limited by the degree to which pharmacokinetics of maternal/fetal are understood. It is well established that melatonin can cross the placenta (47), and the half-life of melatonin is known to be short (~40 min) (18). However, full knowledge of melatonin absorption and effectiveness within the developing fetus is not known. We have shown previously that melatonin is neuroprotective (31), and in the current study we show that it is not able to correct aberrant lung development; however, we cannot report impacts on other organs. Therefore, additional studies, including further elucidation of dosing and timing of administration, are required.

Conclusion

Our study demonstrates that chronic placental insufficiency and subsequent FGR during late gestation in fetal sheep result in alveolar simplification after birth. Melatonin administration did not improve the structure of the growth-restricted lung, nor did it further adversely affect lung development. Melatonin is currently the subject of clinical trials as a neuroprotective therapy in FGR (2, 15), and our study supports its use, with no detrimental effects on the lung observed. However, further studies are required to determine whether it is possible to optimize lung development in growth-restricted fetuses and newborns to reduce the short- and long-term pulmonary consequences of FGR. Given the apparent benign effect of chronic melatonin administration on lung development, our findings support the use of maternal administration of melatonin for reducing fetal and newborn oxidative stress in pregnancies complicated by FGR.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


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