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Antenatal retinoic acid does not alter alveolization or postnatal lung function in preterm sheep

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Antenatal retinoic acid does not alter alveolization or postnatal lung function in preterm sheep. K.E. Willet, A.H. Jobe, M. Ikegami, J. Newnham, P.D. Sly. ©ERS Journals Ltd 2000.

ABSTRACT: Retinoic acid exposure has been shown to promote surfactant production in foetal rats and to promote alveolization in neonatal rats. It was hypothesized that antenatal retinoic acid treatment would promote alveolization and accelerate functional maturation in the lungs of late gestation preterm sheep.

Foetuses received a single *i.m.* injection of all-*trans* retinoic acid (RA, 20 mg·kg^{-1}) or vehicle control at 115 days gestation (term=150 days) and were delivered at 125 days gestation. To examine the longer term effects of RA on alveolization a second group of animals received RA or vehicle at 121 days gestation and were delivered at 146 days gestation.

Liver retinol levels at time of delivery were 2–3-fold higher in both preterm and near-term RA treated animals, indicating a significant impact of RA treatment on retinol metabolism. Dynamic compliance, gas exchange, lung gas volume and saturated phosphatidylcholine pool size at 125 days were unaffected by antenatal RA treatment. Alveolar volume, wall thickness and number at 125 or 146 days were also unaffected by RA treatment.

Retinoic acid, as administered in this study, does not appear to accelerate structural or functional maturation of the foetal sheep lung. Response to retinoic acid may be species dependent, highlighting a need for caution when interpreting results from animal based studies.

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Lung immaturity resulting in respiratory distress syndrome after preterm birth is a frequent cause of neonatal morbidity and mortality [1]. Numerous antenatal therapies aimed at accelerating functional maturation of the foetal lung prior to delivery have been evaluated. To date only glucocorticoids have shown consistent clinical benefits [2]. Antenatal glucocorticoids have multiple effects on the preterm lung that include maturation of the surfactant system [3, 4] and effects on lung structure [5, 6]. Several clinical and animal based studies suggest that retinoic acid and/or retinol (vitamin A), its metabolic precursor, may also play an important role in lung development and maturation. Maternal retinol is transferred from mother to foetus during pregnancy, particularly during the final trimester [7, 8]. Infants born prematurely forego this third trimester transfer of retinol and may have low retinol stores at birth [9]. Retinol deficiency has been implicated in the development of bronchopulmonary dysplasia (BPD) in preterm neonates. Postnatal vitamin A supplementation may improve retinol status and decrease the incidence of BPD [10], although this finding is inconsistent [11, 12]. Antenatal exposure to retinoic acid increases surfactant phospholipid content in foetal rats [13] and stimulates transcription and synthesis of surfactant apoprotein (SP)-B in cultured respiratory epithelial cells [14]. In neonatal rats, retinoic acid prevents the inhibition of septation caused by glucocorticoid exposure during alveolization, and increases alveolar number when given alone [15].

This study was designed to examine whether antenatal retinoic acid treatment would accelerate structural and functional maturation of the lungs of preterm sheep, and also to examine whether any maturational effects persisted until term. It was hypothesized that a single high dose of retinoic acid, given as a slow release preparation, would accelerate maturation of the surfactant system and promote alveolar formation, resulting in an improvement in postnatal lung function.

Methods

Foetal treatment

Protocols were approved by the Animal Ethics Committees at the Children's Hospital Medical Center in Cincinnati (OH, USA) and the Western Australian Dept of Agriculture. Date bred Merino ewes were randomized to either control or treatment groups. Foetuses were delivered at either 125 days (D125) gestation (preterm, n=18) or at 146 days (D146) gestation (near-term, n=14). Those foetuses randomized to be delivered at D125 received a single ultrasound guided i.*m.* injection of 2 mL 10 mg·mL^{-1} all-*trans* retinoic acid (Sigma, St Louis, MO, USA 20 mg·kg^{-1}

based on foetal weight of 2 kg) in sterile olive oil or vehicle control at 115 days gestation. Those foetuses randomized to be delivered at D146 were injected at 121 days gestation. Retinoic acid was shielded from light at all times and solutions were prepared on the day of injection.

For delivery each ewe was sedated with ketamine (1 g *i.m.*) followed by spinal anaesthesia (2% lignocaine, 3 mL). For preterm delivery, the foetal head was exposed through midline abdominal and uterine incisions and the foetus was sedated (10 $mg \cdot kg^{-1}$ ketamine). After administering local anaesthetic (2% lignocaine, *s.c.*) a tracheotomy was performed and a 4.0 mm endotracheal tube secured in place. Lung liquid was removed by suction through the endotracheal tube. Animals were delivered and the umbilical cord cut. Foetuses delivered at D146 were tracheotomized and after removing foetal lung fluid the endotracheal tube was immediately clamped to minimize the possibility of air being drawn into the lungs. A lethal dose of pentobarbital was administered prior to delivery. The lungs were then degassed by allowing any residual air to be absorbed over a 5-min period.

Postnatal measurements

Preterm animals were ventilated for 40 min following delivery to evaluate the effect of retinoic acid on postnatal lung function. Following delivery, lambs were weighed, dried and covered with plastic wrap to minimize heat loss. Temperature was maintained at 39°C by an overhead warmer. Animals were placed on an infant ventilator (BP200; Bournes Riverside, CA, USA) set to deliver 100% oxygen at a rate of 40 $breaths \cdot min^{-1}$, inspiratory time 0.75 s and positive end expiratory pressure 3 cmH_2O. Peak inspiratory pressure (PIP) was initially set at 35 cmH_2O. Both tidal volume and arterial carbon dioxide partial pressure (P_{a,CO_2}) were monitored closely and PIP was adjusted to maintain adequate ventilation. No other ventilator setting was altered during the study. In order to minimize the risk of ventilator-induced lung injury PIP was not permitted to exceed 40 cmH_2O. An arterial catheter was advanced to the level of the descending aorta *via* an umbilical artery and lambs were anaesthetized by slow arterial infusion of pentobarbital sodium (15 $mg \cdot kg^{-1}$).

A pressure transducer (model 8507C-2; Endevco, San Juan Capistrano, CA, USA) and pneumotachograph (model 35-597; Hans Rudolph, Kansas City, KS, USA) were placed between the tracheostomy tube and the ventilator to measure tracheal pressure (P_{tr}) and flow ($\dot{V}$) respectively. Volume (V) was obtained by integrating flow. Compliance was measured at 10 min intervals using multiple linear regression analysis of P_{tr}, $\dot{V}$ and V signals [16]. Arterial oxygen partial pressure (P_{a,O_2}) and P_{a,CO_2} were measured at 10 min intervals. Target P_{a,CO_2} was 6.0–6.7 kPa (45–50 mmHg), however animals were permitted to become hypercarbic when target P_{a,CO_2} was not able to be attained at maximum PIP (40 cmH_2O). At 40 min postdelivery animals were given a lethal dose of pentobarbital sodium. The lungs were degassed firstly by compression of the thoracic cavity then by clamping the endotracheal tube allowing the remaining oxygen to be absorbed over a 5-min period. The chest was opened and lung gas volume at a pressure of 40 cmH_2O was determined. Left lungs were lavaged with iced saline. Alveolar lavage surfactant pool size was estimated by isolating saturated phosphatidylcholine (SatPC) from lipid extracts [17] and quantifying inorganic phosphorus [18]. Lung tissue SatPC was similarly estimated on a weighed sample of lavaged lung tissue. Near-term animals were not ventilated following delivery. In these animals the chest was opened and lung volume at 40 cmH_2O was determined.

Morphometry

Morphometric assessment was performed on all D125 and D146 animals. As lung maturation is known to vary between regions of the lung [19] all morphometric assessments were performed on the right cranial lobe. The right cranial lobe was fixed overnight at a pressure of 30 cmH_2O *via* bronchial instillation of 10% phosphate-buffered formalin. Fixed lobe volume (FLV) was measured by volume displacement [20]. Each lobe was then cut into 5-mm serial slices and three slices were randomly chosen for morphometric examination [21]. Measurements were obtained from three 5-μm haematoxylin and eosin-stained sections per lobe.

Stained sections were enlarged and printed onto photographic paper at a magnification of ×16. Volume fraction of lung parenchyma (PF=alveoli and alveolar ducts), nonparenchyma (conducting airways plus blood vessels), interlobular septa (forming the distinct lobulation of the lungs) and pleura were estimated by superimposing a linear point counting grid (464 lines/928 points) onto each of the photographic images. The volume fraction is equal to Pi/Pt, where Pi represents the number of test points hitting the structure of interest (*e.g.* parenchyma) and Pt is the total number of points hitting the reference space (total of all compartments).

Digitized images from 10 nonoverlapping parenchymal fields were captured from each 5-μm section using a Sony 3CCD Color Video Camera (Sony, Tokyo, Japan) interfaced with a Leica DMLS Microscope (Leica, Heerbrugge, Switzerland) and a Macintosh 8100/80AV computer (Cupertino, CA, USA). Images were examined at a final magnification of ×950. The number of points which fell on airspace and on alveolar septal tissue and the number of air/tissue tissue/air intercepts were counted by superimposing a linear point counting grid (21 lines/42 points). The mean linear intercept of alveoli and alveolar ducts (MLI) was determined using the formula $MLI = 2\ L_T / I_O$ where L_T is the length of the test line within the reference volume and I_O is the number of intercepts with the air tissue interface [21]. Alveolar wall thickness (TD) was determined as volume per unit area of alveolar surface according to the formula $TD = (Pi/Pt \times MLI)/4$ where Pi/Pt is equal to the volume fraction of alveolar septa [21]. Alveolar number per unit volume (N_V) was calculated according to the method of Weibel [22] using the equation:

$$N_V = N_A{}^{1.5}/(BAF^{0.5})D \qquad (1)$$

where N_A is the number of alveoli per unit area, AF the volume fraction of alveoli, both determined on digitized images, B the shape constant describing alveolar shape (1.55) and D the distribution variable of the characteristic linear dimension of the alveoli [1]. In transverse section, alveoli were identified as those structures opening onto a

common airspace (alveolar duct). In cross section, alveoli were identified on the basis of size, shape and morphology. A total of 200–400 alveoli per animal were counted. Ambiguous structures were rejected. In practice <2% of the total number of structures encountered were rejected. Theoretically the proportion of rejected structures that are alveoli may differ between control and RA treated animals, leading to errors, but given the very small number of excluded structures, the differences would have to be marked to significantly affect the calculated morphometric parameters. Total number of alveoli in the right upper lobe was calculated by multiplying lobe volume by N_V. Average alveolar volume was calculated by dividing total alveolar volume (FLV×PF×AF) by total number of alveoli.

Retinol measurements

Cord blood samples were collected at time of delivery and total plasma retinol (*i.e.* retinol + retinyl esters) concentration was determined by column chromatography and fluorometric analysis according to the method of THOMPSON *et al*. [23]. Weighed samples of lung and liver were collected and snap frozen for later analysis. Lipid fractions were extracted and retinol concentrations were determined by direct fluorometric analysis [23].

Statistical analyses

Group differences were examined by two-way analysis of variance, to determine changes with treatment and gestational age. Statistical significance was accepted at $p< 0.05$.

Results

Foetal growth

Both body and lung weights increased substantially between D125 and term (table 1). Retinoic acid treatment did not affect foetal growth, whether delivery was preterm or at term. There was also no evidence that retinoic acid exposure caused an increase in the rate of foetal deaths. Of interest, all but two of the animals delivered at D125 after retinoic acid treatment had open eyes, while eyes remained closed in all control animals ($p<0.01$).

Table 1. – Mortality rate, birth weight, wet lung weight and excised lung gas volume at 40 cmH_2O in near-term and preterm foetuses

Group	M/F	Foetal death	Body weight kg	Lung weight g	Lung volume mL·kg^{-1}
D125 control	5/3	0/8	2.7±0.1	105.3±4.0	14.2±1.6
D125 retinoic acid	6/4	0/10	2.6±0.1	98.6±6.2	14.9±1.6
D146 control	4/3	1/8	4.9±0.2*	185.9±9.6*	41.2±2.2*
D146 retinoic acid	3/4	1/8	5.0±0.3*	171.1±9.2*	33.1±5.6*

Data represent group mean±SEM. M: male; F: females; D125: 125 days of gestation; D146: 146 days of gestation. *: $p<0.05$ *versus* D125 control and D125 retinoic acid groups.

Table 2. – Postnatal lung function, alveolar and lung tissue saturated phosphatidylcholine concentration and tissue retinol levels

	Control	Retinoic acid
D125		
Compliance mL·cmH_2O·kg^{-1}	0.15±0.02	0.17±0.02
P_{a,O_2} mmHg	72±13	49±8
P_{a,CO_2} mmHg	99±9	98±9
Alveolar SatPC µmol·kg^{-1}	0.39±0.05	0.37±0.06
Lung tissue SatPC µmol·kg^{-1}	60±3	58±7
Lung retinol µg·g^{-1}	0.30±0.40	0.33±0.30
Liver retinol µg·g^{-1}	3.40±0.54	6.40±0.81*
Plasma retinol µg·g^{-1}	0.19±0.01	0.17±0.02
D146		
Lung retinol µg·g^{-1}	0.20±0.20	0.14±0.30
Liver retinol µg·g^{-1}	1.86±0.54	6.42±1.00*
Plasma retinol µg·g^{-1}	0.11±0.04	0.11±0.02

Data represent group mean±SEM. D125: 125 days of gestation; P_{a,O_2}: arterial oxygen partial pressure; P_{a,CO_2}: arterial carbon dioxide partial pressure; SatPC: saturated phospabtidylcholine; D146: 146 days of gestation. *: $p<0.05$ *versus* relevant control group. 1 mmHg = 0.133 kPa.

Postnatal lung function

There was no evidence of an improvement in compliance, lung gas volume or gas exchange in preterm retinoic acid treated animals (tables 1 and 2). Lung gas volume was also similar in control and treated animals delivered near term (table 1). Neither alveolar nor lung tissue SatPC was increased in the preterm lung by retinoic acid treatment (table 2).

Lung morphometry

Fixed lobe volume increased significantly between D125 and D146 (table 3). The volume fraction of parenchyma increased and the volume fractions of interlobular septa and pleura decreased during this period. Retinoic

Table 3. – Lung morphometry

	D125		D146	
	Control	Retinoic acid	Control	Retinoic acid
FLV mL	32.1±3.2	30.9±3.0	58.3±5.0*	53.2±4.3*
PF %	66.2±2.8	68.1±2.6	83.1±2.0*	83.4±1.2*
NPF %	18.2±1.8	15.5±1.2	12.2±1.6	12.3±1.3
ISF %	12.6±2.0	13.6±1.8	3.3±0.7*	3.0±0.8*
PLF %	2.9±0.2	2.8±0.4	1.4±0.3*	1.2±0.2*
$N_V \times 10^6$·cm^{-3}	21.5±2.3	18.3±2.0	13.6±1.0*	14.5±1.4*
$N_T \times 10^6$	454±62	386±62	646±52*	639±60*
$V_A \times 10^3$ µm^3	13.3±1.4	15.4±1.8	31.7±2.8*	28.2±1.8*
TD µm	4.08±0.33	3.94±0.13	2.30±0.05*	2.55±0.06*
MLI µm	45.9±2.3	48.3±2.2	46.7±1.8	48.8±0.9

Data represent group mean±SEM. D125: 125 days of gestation; D146: 146 days of gestation; FLV: fixed lobe volume; PF: parenchymal fraction; NPF: nonparenchymal fraction; ISF: interlobular septal fraction; PLF: pleural fraction; N_V: numerical density of alveoli; N_T: total number of alveoli in right upper lobe; V_A: average alveolar volume; TD: mean alveolar wall thickness; MLI: mean linear intercept. *: $p<0.05$ *versus* D125 control and D125 retinoic acid groups.

acid treatment did not affect volume or volume fractions at D125 or at D146 (table 3).

Alveoli in D125 animals were typically shallow, thick walled structures (fig. 1a). As a result of elongation and attenuation of interalveolar septa, alveoli in near-term animals were usually deep, thin walled structures (fig. 1b). This process coincided with a doubling in average alveolar volume and a 30% decrease in numerical density, *i.e.* number of alveoli per unit volume (table 3). Total alveolar number increased by >50% and mean alveolar wall thickness decreased by ~40% during this period (table 3). Mean linear intercept did not change. Retinoic acid treatment did not affect alveolar size, wall thickness or number in either preterm or near-term animals (table 3, fig. 1).

Retinol levels

Retinol levels were highest in liver, intermediate in lung tissue and lowest in plasma. Concentrations were similar for term and preterm animals (table 2). Liver retinol levels were significantly higher in retinoic acid treated animals both at D125 and at D146, although plasma and lung tissue retinol levels were unaffected.

Discussion

This study examined the impact of antenatal retinoic acid treatment on lung structure and function in preterm and near term sheep. For animals delivered at D125, the objective was to evaluate the impact of structural changes on lung function by examining the effects of retinoic acid exposure on surfactant production and alveolar formation. For animals delivered near term, the primary objective was to evaluate any long term effects of retinoic acid treatment on alveolar structure. Retinoic acid treated animals had 2–3-fold higher liver retinol levels whether delivered prematurely or near term, indicating that a dose of 20 $mg \cdot kg^{-1}$ had a significant impact on foetal retinol metabolism. No evidence of accelerated structural or functional maturation of the foetal lung following antenatal retinoic acid treatment was found.

The results are at odds with previous studies which have shown that retinoic acid can stimulate the surfactant system [13, 14, 24]. SP-B levels are significantly increased in both human pulmonary adenocarcinoma cells (H441) and human foetal lung explants cultured in the presence of retinoic acid [14, 24]. Retinol deficiency during pregnancy leads to significantly reduced lung phosphatidylcholine concentration in neonatal rats [25]. Conversely, antenatal administration of retinoic acid upregulates surfactant production [13]. In this study a single dose of retinoic acid (0.1, 1 or 10 $mg \cdot kg^{-1}$) was administered by maternal intragastric injection at 16 days gestation. When foetuses were delivered at 18 days gestation, increases in lung phosphatidylcholine were observed in foetuses exposed to 1 or 10 $mg \cdot kg^{-1}$ retinoic acid. This 2 day interval of treatment to effect is consistent with studies which show that glucocorticoids also enhance surfactant synthesis within 2–3 days of treatment in the rat [26, 27]. A considerably longer treatment to delivery interval (10 or 25 days) was chosen for the present study: based on

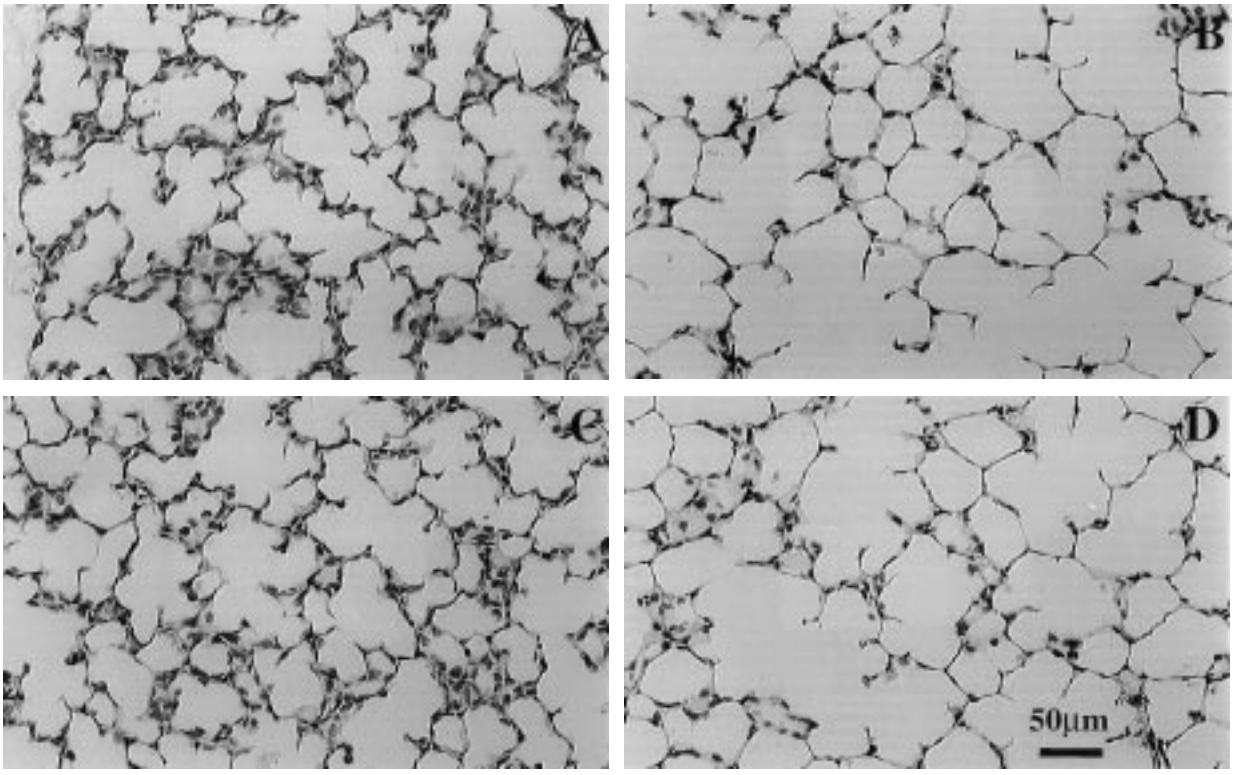

Fig. 1. – Lung parenchyma: 5 µm haematoxylin and eosin stained sections. A) 125 days of gestation (D125) control animal; B) 146 days of gestation (D146) control animal; C) D125 retinoic acid treated animal; D) D146 retinoic acid treated animal. Elongation and attenuation of interalveolar septa with advancing gestation results in larger, thinner walled alveoli. Retinoic acid treatment did not alter alveolar morphology. Original magnification ×315.

previous observations in sheep, a longer treatment to delivery interval is appropriate in this species. Antenatal glucocorticoids increase alveolar surfactant pool size 7 days after exposure, with little impact prior to this time [4]. Increased surfactant pool size is still evident up to 21 days after a single maternal dose of betamethasone [28]. The lack of effect of retinoic acid treatment on the surfactant system in preterm sheep suggests that responsiveness may be species dependent.

In addition to effects on the surfactant system, a recent study by MASSARO and MARRARO [15] reported that retinoic acid promotes alveolization in neonatal rats. There are several differences between the current study and the study by MASSARO and MARRARO [15] in rats. Firstly, the current study animals were exposed to retinoic acid *in utero*, while MASSARO and MARRARO [15] examined the impact of postnatal exposure. Alveolization is an entirely postnatal process in the rat, beginning at around day 4 [29], therefore it is appropriate in this species to examine the influence of therapeutic interventions during the postnatal period. Retinol stores increase greatly in the lungs of foetal rats in the final third of gestation [30]. Depletion of these stores begins just prior to birth and continues postnatally during the first few days, and it is thought that the developing lung may depend on these local stores for cell proliferation and differentiation during alveolization. In contrast, alveolization begins prenatally in foetal sheep at around 120 days (80%) gestation [31], so it is appropriate in this species to examine therapeutic interventions *in utero*. In this species, as in humans [9], maternal retinol is probably the primary source of retinoic acid during the early period of alveolization. Therefore the disparity between the current results in sheep and previous findings in rats may relate to differences in the primary source of endogenous retinoids during alveolization (foetal *versus* maternal).

A second difference between the current study and that of MASSARO and MARRARO [15] relates to treatment schedule: the current authors administered retinoic acid as a single, slow-release injection rather than in daily injections. It is both hazardous and impractical to administer daily foetal injections, therefore the authors chose to administer a single, large dose. The single dose of 20 $mg \cdot kg^{-1}$ was considerably higher than the 6 $mg \cdot kg^{-1}$ total dose (500 $\mu g \cdot kg^{-1}$ daily injections between postnatal days 3 and 14) used in the study by MASSARO and MARRARO [15]. In the present study, lung retinol levels were similar in control and retinoic acid treated lambs 10 days after treatment, suggesting that a single dose may not be sufficient to promote sustained high lung retinol levels. By contrast, in rats, a single dose of retinyl palmitate by maternal intragastric administration on gestational day 16 leads to a significant increase in foetal lung retinyl esters within 24 h, an effect which persists until postnatal day 14 [32]. These observations suggest that rats and sheep may differ with regard to lung retinol metabolism, and that a single high dose of retinoic acid may not impact on alveolization in sheep.

Although a sustained high lung retinol level was not observed, two important observations suggest that the experimental approach was indeed sufficient to elicit developmental changes in responsive organs. Firstly, liver retinol levels were 2–3-fold higher in retinoic acid treated animals than in their control counterparts both 10 and 25 days after treatment. Retinoic acid is an end product which cannot enter other retinol pools, therefore the high levels of liver retinol do not reflect uptake of exogenous retinoic acid. The most plausible explanation for such high levels is a sparing effect on hepatic retinol stores as a result of the addition of readily available retinoic acid for development of lung and other responsive organs. A second observation, which clearly demonstrates that a single dose was sufficient to exert developmental effects on responsive foetal organs, was the presence of open eyes in seven of the nine retinoic acid treated animals delivered at D125. This points to precocious keratinization of skin of the eye lids in retinoic acid treated foetuses, as eye opening is not usually seen until around 135 days gestation in foetal sheep (A.H. Jobe, unpublished observation).

A final consideration which may account for the difference between the present results and previous findings in rats, is the method by which alveolar number and volume were estimated. While the present study determined alveolar number and volume by 2-dimensional analysis on 5 μm sections [33], MASSARO and MARRARO [15] estimated these parameters on serially reconstructed sections. In essence, the principal advantage of serial reconstruction over 2-dimensional analysis is its ability to definitively differentiate alveoli from alveolar ducts. However, it is considerably more labour intensive, and as a result, considerably fewer alveoli are sampled (30 *versus* 200–400 per animal). Misclassification of alveolar ducts as alveoli would almost certainly reduce the sensitivity of 2-dimensional analysis to detect changes in alveolar number and/or volume, although the magnitude of this effect is unknown, as these two techniques have never been directly compared. Serial reconstruction is reportedly more sensitive to changes in airspace size than the mean linear intercept method [34]. The mean linear intercept provides an estimate of the size of all airspaces (*i.e.* alveoli and alveolar ducts), therefore its ability to detect changes in alveolar size may be relatively low. In the present study it was also found that the mean linear intercept method failed to detect an increase in alveolar size between D125 and D146, while 2-dimensional analysis indicated a two-fold increase in this parameter. Clearly 2-dimensional analysis provides a more sensitive indicator of changes in alveolar size than does the mean linear intercept method.

While the present study demonstrated that antenatal retinoic acid treatment did not impact on foetal lung maturation during normal development, its impact on betamethasone induced inhibition of septation was not examined. Both *in vitro* and *in vivo* studies have demonstrated an antagonistic effect of retinoic acid and glucocorticoids on lung maturation [15, 35]. A recent study by WHITNEY *et al*. [36] suggests that cellular retinol binding protein I (CRBP-I) may play an important role in retinoic acid induced septation of airspaces, and that inhibition of septation by glucocorticoids is due to down-regulation of CRBP-I. Although antenatal retinoic acid treatment did not promote alveolar septation in foetal sheep, the data do not preclude the possibility that concurrent administration of betamethasone and retinoic acid, either pre- or postnatally, could prevent the inhibition of septation induced by betamethasone.

The data contrast sharply with previous findings in rats, and highlight a very important issue: responsiveness to retinoic acid may be species dependent. In rats lung retinyl

esters peak between day 16–19 of gestation, fall just prior to term and remain low between postnatal day 2 and 14 [30]. Surfactant synthesis is evident from around gestational day 18 [37], when lung retinyl levels are highest, whereas alveolization occurs postnatally, when lung retinyl levels are low. Despite the almost five-fold differences in endogenous lung retinyl levels during the two developmental stages, both alveolization and surfactant synthesis are responsive to supplemental retinoids. Both acute and chronic exposure to retinoic acid are effective, and foetal rats appear to respond to significantly lower doses than foetal sheep. A single maternal oral dose of just 1 $mg \cdot kg^{-1}$ all-*trans* retinoic acid was sufficient to elicit an increase in lung phosphatidylcholine in gestational day 18 rat foetuses [13]. Given that placental transfer of retinoic acid is relatively inefficient [8], a maternal dose of 1 $mg \cdot kg^{-1}$ would result in a foetal dose of considerably less. Almost all previously published studies examining the effects of retinoids on lung development have been in the rat. Repetitive daily treatment with 500 $\mu g \cdot kg^{-1}$ retinoic acid has been reported to increase alveolar number in genetically modified mice with severe emphysema [34], but there are currently no published data in other animals. The findings highlight the need for further studies on the effects of retinoic acid on lung development in animal models other than the rat, and suggest that caution should be used when extrapolating results from animal based studies to humans.

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