Lack of response to all-trans retinoic acid supplementation in adult dogs following left pneumonectomy

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Submitted 10 May 2005; accepted in final form 12 June 2005

Yan, Xiao, Dennis J. Bellotto, D. Merrill Dane, R. Geoffrey Elmore, Robert L. Johnson, Jr., Aaron S. Estrera, and Connie C. W. Hsia. Lack of response to all-trans retinoic acid supplementation in adult dogs following left pneumonectomy. J Appl Physiol 99: 1681–1688, 2005.—We showed previously that removing 55–58% of the lung by right pneumonectomy (R-PNX) in adult dogs triggers compensatory growth of the remaining lung, but removing 42–45% of the lung by left PNX (L-PNX) does not. We also showed that, following R-PNX, supplemental all-trans retinoic acid (RA) selectively enhances alveolar capillary endothelial cell volume (Yan X, Bellotto DJ, Foster DJ, Johnson RL, Jr., Hagler HH, Estrera AS, and Hsia CC. J Appl Physiol 96: 1080–1089, 2004). We hypothesized that RA supplementation might enhance compensation following L-PNX and tested this hypothesis by administering RA (2 mg·kg−1·day−1, 4 days/wk) or placebo orally to litter-matched adult foxhounds for 4 mo following left PNX. Resting lung function was measured under anesthesia. Air and tissue volumes of the remaining lung were assessed by high-resolution computed tomography scan and by detailed postmortem morphometric analysis of the fixed lung. There was no significant difference in resting lung function, lung volume, alveolar structure, or septal ultrastructure between RA and placebo treatment groups. We conclude that RA supplementation does not induce post-PNX compensatory lung growth in the absence of existing cellular growth activities initiated by other primary signals.

compensatory lung growth; lung resection; alveolar capillary surface area; lung diffusing capacity; high-resolution computed tomography; lung morphometry

PNEUMONECTOMY (PNX) is a robust model of restrictive lung disease that has been used to examine the mechanisms of compensatory lung growth and therapeutic approaches of growth enhancement. Following PNX, a greater mechanical strain imposed on the remaining lung constitutes the primary signal that triggers a spectrum of adaptive responses, including 1) recruitment of existing physiological reserves, 2) remodeling of the remaining lung structure, and 3) reinitiation of alveolar tissue growth (12). These adaptive responses significantly increase diffusing capacities of the remaining lung and optimize aerobic capacity. In adult dogs, post-PNX compensatory lung growth is evident only when the strain imposed on the remaining lung exceeds a certain threshold, that is, following 55–58% lung resection by right PNX but not following 42–45% resection by left PNX (9, 10, 26).

One important question is whether compensatory lung growth could be pharmacologically enhanced in such a way as to improve lung function. An example is all-trans retinoic acid (RA), which regulates many aspects of lung development, differentiation, growth, and repair. In rodents, RA promotes neonatal alveolar formation (21), rescues failed alveolar septation (23), ameliorates the alveolar loss due to emphysema (22), and enhances post-PNX lung growth (15). In previous studies, we supplemented the diet of adult dogs with RA following right PNX where active compensatory alveolar growth normally occurs. RA supplementation during the early post-PNX period selectively enhances ongoing compensatory growth of alveolar endothelial cells and capillaries without enhancing the growth of other septal cell types (45). RA supplementation also causes distortion of septal ultrastructure and alveolar architecture, thereby preventing a net increase in the absolute alveolar surface area or lung diffusing capacity during the treatment period (7, 45). These results illustrate the need for a balanced tissue response at all levels of organization if optimal physiological improvement is to be realized.

Our next question was whether exogenous RA supplementation could reinitiate compensatory alveolar growth of the mature lung in the absence of existing cellular growth activities. To address this issue, we administered supplemental RA to adult dogs for 4 mo following left PNX, where adaptation normally occurs via recruitment of physiological reserves and remodeling without invoking the generation of new alveolar tissue. If RA is an independent signal for compensatory lung growth, we expected to find larger alveolar septal cell volumes and surface areas and possibly improved function in the remaining lung of RA-supplemented animals compared with placebo controls. Lung function was assessed at rest under anesthesia. Air and tissue volumes of the remaining lung were measured by high-resolution computed tomography (CT) scan, and detailed morphometric analysis of alveolar ultrastructure was performed postmortem.

METHODS

Animal procedures. The Institutional Animal Care and Use Committee approved all procedures. An experimental flow chart is shown in Fig. 1. The study design was identical to that previously described for right PNX (7, 45). Twelve litter-matched male foxhounds underwent left PNX at ~1 yr of age. The procedure for surgery and drug administration has been described previously (45). Dogs were fed a standard unrestricted diet. Beginning 1 day following left PNX, six animals received all-trans RA (Sigma, 2 mg/kg orally, dissolved in vegetable oil), while six littermates received placebo (vehicle only). The dissolved drug or placebo was mixed with honey and peanut

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butter and fed into the dog’s mouth once daily, 4 days/wk over 4 mo. The animal was observed until the mixture had been swallowed completely. A treatment holiday was provided 3 days/wk to minimize the induction of drug metabolism. This method of administration was kindly suggested by Drs. Donald Massaro and Gloria Massaro (Lung Biology Laboratory, Georgetown University, Washington, DC). Oral all-trans RA is rapidly absorbed. We have previously shown that this dose resulted in accumulation of RA in lung tissue and significant biochemical effects (45). The dose was below that reported to cause toxicity in dogs (5–10 mg/day).

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Physiological studies. During the last month of treatment, lung function was measured supine under anesthesia by established methods (7). The animal was anesthetized, intubated, and mechanically ventilated to suppress spontaneous ventilation. Esophageal and mouth pressures, rectal temperature, heart rate, and transcutaneous oxygen saturation were monitored. The endotracheal tube was connected via a manifold to either the ventilator or a large calibrated syringe containing the desired inspiratory gas mixture. Before each measurement, the animal was given three cumulative tidal breaths (total 45 ml/kg) to fully expand the lungs, followed by passive deflation to EELV, the test mixture was delivered to the lung and rebreathed in and out of the syringe for 16 s at 30 breaths/min. Gas concentrations were continuously monitored at the mouth by a chemiluminescence analyzer for NO (model 280, Siemens Instruments, Boulder, CO), an infrared gas analyzer for CO, methane, and acetylene (Sensors, Saline, MI), and a mass spectrometer for O2, CO2, and N2 (Perkin Elmer 1100). Analyzers were calibrated each day according to the manufacturer’s specification. All signals were digitized. Rebreathing measurements were obtained at two inspired O2 concentrations (21 and 99% O2) at two lung volumes (30 and 45 ml/kg above EELV) given in random order. Duplicate measurements under each condition were averaged.

A venous blood sample was drawn before and at the end of the experiment and analyzed for hemoglobin, carboxyhemoglobin, and methemoglobin concentrations (OSM3, Radiometer, Copenhagen, Denmark). The instrument was calibrated for dog blood. Hematocrit was measured by using a capillary tube centrifuge.

Lung volumes (BTPS) were calculated from methane dilution. Cardiac output was calculated from the exponential disappearance of end-tidal acetylene with respect to methane, corrected for the intercept of CO disappearance (28). DLCO and DLNO were calculated from the exponential disappearance of CO and NO, respectively, with respect to methane (27, 34). End-tidal points were selected from the log linear portion of the disappearance curves. From DLCO measured at two levels of alveolar O2 tension (P(AO2)), we estimated membrane diffusing capacity for CO (DM(CO)) and pulmonary capillary blood volume (Vc) using the Roughton-Forster relationship:

\[
\frac{1}{\text{DL}_{CO}} = \frac{1}{\text{DM}_{CO}} + \frac{1}{\theta_{CO} \cdot Vc}
\]

where \(\theta_{CO}\) is the empirical rate of CO uptake by dog whole blood at 37°C (in ml CO/min·1·1·mmHg−1·ml blood−1) calculated from the mean P(AO2) (in Torr) during rebreathing and the hemoglobin concentration ([Hb]) in g/dl:

\[
\frac{1}{\theta_{CO}} = (0.929 + 0.00517 \cdot P(AO2)) \cdot \frac{14.6}{[Hb]}
\]

Estimates of DM(CO) and Vc were then used to calculate the DLCO expected under standardized conditions (DLCO-std), i.e., at a constant P(AO2) = 120 Torr and [Hb] = 14.6 g/dl. Measurement of DLCO is equally sensitive to resistances imposed by the alveolar membrane and erythrocytes, whereas DLNO predominantly reflects the resistance imposed by alveolar membrane, as the erythrocytes’ resistance to NO uptake is small relative to the resistance of the membrane (1, 34). The effective septal volume for gas exchange was estimated from the extrapolated intercept of acetylene disappearance to time 0. Estimates of septal volume from all rebreathing maneuvers in a given animal were averaged.

CT scan. On a separate day, high-resolution CT scan (GE High Speed CTI) of the lung and thorax was performed under propofol anesthesia. The animal was intubated with a cuffed endotracheal tube, placed supine, and mechanically ventilated to suppress spontaneous breathing. Before imaging, the lungs were hyperinflated with three cumulative tidal breaths followed by passive expiration to EELV. The lungs were inflated to a volume that had been previously determined to yield a transpulmonary pressure of 20 cmH2O. The breath was held for 40–45 s during scanning. Airway pressure was continuously monitored. During breath hold, O2 is consumed and CO2 is produced, and net volume loss was minimal. A scout film was first obtained, followed by volumetric CT imaging from the lung apex to the

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the costophrenic angle at 3 × 3-mm collimation; images were reconstructed at consecutive 1-mm intervals, resulting in ~300 images per animal.

Using Object-Image version 1.62 (a public domain program based on NIH Image by Norbert Vischer, University of Amsterdam, Amsterdam, the Netherlands), the area occupied by lung in each image was obtained by density thresholding and manual tracing. Conducting airways and blood vessels larger than 1–2 mm were excluded by density threshold. The area occupied by lung in each image was multiplied by slice thickness to obtain volume; total lung volume was summed from the volume from individual images. Because the average CT density of lung is directly proportional to the ratio of tissue to air, the CT density (in Houndsfield units) of tracheal air and muscle was used to partition total lung volume into air and tissue volumes by established methods (7).

**Terminal procedure.** At the end of treatment, animals were fasted overnight, premedicated (atropine 0.05 mg/kg sc), anesthetized (pento-barbital 25 mg/kg iv), intubated with auffed endotracheal tube via a tracheostomy, and mechanically ventilated (12 ml/kg tidal volume, 12 breaths/min). The abdomen was opened via a midline incision. The ventilator was disconnected, and a rent was made through each

1. **Lung volume.** The fixed right lung was divided into upper and lower strata. The upper stratum consisted of the upper and middle lobes, which were often incompletely separated. The lower stratum consisted of the lower and cardiac lobes. Volume of the intact stratum was measured by saline immersion (41), with the clamps in place to maintain airway pressure. Then each stratum was sliced serially at 2-cm intervals. The face of each section was photographed for volume estimation by point counting using the Cavalieri principle (24, 46); this volume reflects the state of the tissue free from tension and was used in subsequent morphometric calculations.

**Sampling, processing, and morphometric analysis.** These procedures are well established (45). A four-level stratified analytical scheme was employed: gross (level I, about ×2), low-power light microscopy (level II, ×275), high-power light microscopy (level III, ×550), and electron microscopy (EM; level IV, ×19,000) (39). For level I, images of 2-cm serial sections were analyzed by point counting using standard test grids to exclude structures >1 mm in diameter, to estimate the volume density of coarse parenchyma with respect to total lung volume. For level II, four blocks were sampled per stratum (8 blocks per dog) by a systematic random scheme, embedded in glycol methacrylate, sectioned (5 μm thick), and stained with toluidine blue. One section per block was overlaid with a test grid (100 × 100 μm) and stained with toluidine blue. One section per block was examined at ×500. From a random start, at least 10 nonoverlapping microscopic fields were systematically sampled. The volume fraction of structures 20 μm to 1 mm in diameter were quantified by point counting to estimate the volume density of fine parenchyma (gas exchange region) with respect to coarse parenchyma.

For levels III and IV, four blocks were sampled per stratum (8 blocks per animal) by a systematic random scheme, postfixed with 1% osmium tetroxide in 0.1 M cacodylate buffer, treated with 2% uranyl acetate, dehydrated through graded alcohol, and embedded in Spurr. Each block was sectioned (1 μm thick) and stained with toluidine blue. One section per block was examined at ×550. From a random start, at least 20 nonoverlapping microscopic fields per block were systematically analyzed by point counting (80 images per stratum) in order to estimate the volume density of alveolar septa with respect to fine parenchyma (level III).

For level IV analysis, 80-mm-thick sections were obtained from two blocks per stratum, mounted on copper grids, and examined under a transmission electron microscope (JEOL EXII) at approximately ×19,000. Thirty nonoverlapping EM fields per grid (60 images per stratum) were sampled systematically from a random start; images were captured with a charge-coupled device camera (Gatan, model C73–0200) and projected onto a Sony high-resolution monitor overlaid with a test grid. Septal cells were identified by their typical morphological characteristics (8). The volume densities of epithelium (types I and II), interstitium, endothelium, capillary blood cells, and plasma with respect to septal volume were estimated by point counting. The alveolar epithelial and capillary surface densities were estimated by intersection counting. At least 200 points or intersections were counted per grid (coefficient of variation <10%). The length of test lines (l) transecting the barrier from epithelial surface to the nearest erythrocyte membrane were measured to calculate the harmonic mean thickness of the tissue-plasma barrier (\( t_{ab} \)):

\[
\tau_{ab} = \frac{1}{magnification \cdot \sum_{i=1}^{n} \frac{1}{l_i}}
\]

**Morphometric lung diffusing capacity.** Diffusing capacities for oxygen (\( D_{O_2} \)) were calculated by an established morphometric model (38, 40) that describes the diffusion path from alveolar air to capillary hemoglobin as two serial conductances through the tissue-plasma barrier (\( D_{bO_2} \)) and within capillary erythrocytes (\( D_{eO_2} \)):

\[
\frac{1}{D_{lO_2}} = \frac{1}{D_{bO_2}} + \frac{1}{D_{eO_2}}
\]

where

\[
D_{bO_2} = K_{bO_2} \cdot \left( \frac{S_m + S_t}{2 \cdot \tau_{ab}} \right)
\]

and

\[
D_{eO_2} = \Theta_{oe} \cdot Vc
\]

where \( S_m \) and \( S_t \) are the measured alveolar and capillary surface areas, respectively; \( K_{bO_2} \) is the Krogh diffusion coefficient for \( O_2 \) in tissue and plasma (5.5 × 10^{-10} cm^2 s^{-1} mmHg^{-1}) (42); and \( \Theta_{oe} \) is the rate of \( O_2 \) uptake by dog whole blood. We used \( \Theta_{oe} = 0.06466 \) ml \( O_2 \cdot ml^{-1} \cdot s^{-1} \cdot mmHg^{-1} \) based on standard equations (33) and average values previously measured in dogs during exercise, i.e., rectal temperature = 40°C, hemoglobin concentration = 19.0 g/dl, and \( P_{A\ O_2} = 100 \) Torr. Estimates of diffusing capacity by this model correlate strongly with that measured by physiological methods at peak exercise (33).

Absolute volume and surface area were calculated by relating the respective volume and surface densities at each level back through the cascade of levels to the volume of the stratum measured by the Cavalieri principle (39). Data were calculated for each stratum separately; then a volume-weighted average was obtained for the entire lung.

**Double-capillary profiles.** Double alveolar-capillary profiles are typical of the developing lung and only infrequently observed in the adult lung. We systematically sampled EM grids at ×1,000 and counted the number of capillary intercepts using the same test grid as in level IV analysis. Each capillary intercepted by a test line was classified as single (only one capillary profile along that portion of the septum) or double (two separate capillary profiles overlap the same portion of the septum). Two grids per animal (one from upper and one from lower stratum) were systematically and completely counted, resulting in over 200 total intercepts per animal. Data were compared with that in separate adult sham dogs and with our published data in...
Table 1. Physiological measurements at rest under anesthesia

<table>
<thead>
<tr>
<th>Volume Above EELV, ml/kg</th>
<th>Placebo</th>
<th>Retinoic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>26.3±1.1</td>
<td>26.0±1.3</td>
</tr>
<tr>
<td>Hemoglobin, g/dl</td>
<td>12.11±0.21</td>
<td>12.21±0.12</td>
</tr>
<tr>
<td>EELV, ml</td>
<td>30 1,744</td>
<td>1,694±79</td>
</tr>
<tr>
<td>EELV, ml</td>
<td>45 2,221±109</td>
<td>2,165±104</td>
</tr>
<tr>
<td>EELV, ml</td>
<td>30 782±60</td>
<td>743±35</td>
</tr>
<tr>
<td>EELV, ml</td>
<td>45 817±64</td>
<td>777±38</td>
</tr>
<tr>
<td>Cardiac output, ml/min</td>
<td>30 3,116±225</td>
<td>3,146±223</td>
</tr>
<tr>
<td>Stroke volume, ml</td>
<td>30 20.4±0.8</td>
<td>21.7±1.3</td>
</tr>
<tr>
<td>Stroke volume, ml</td>
<td>45 21.4±0.7</td>
<td>20.5±0.8</td>
</tr>
<tr>
<td>DLCO-std, ml/min⁻¹·mmHg⁻¹</td>
<td>30 19.08±1.07</td>
<td>17.98±1.19</td>
</tr>
<tr>
<td>DLCO-std, ml/min⁻¹·mmHg⁻¹</td>
<td>45 20.81±0.94</td>
<td>18.44±0.67</td>
</tr>
<tr>
<td>DLNO, ml/min⁻¹·mmHg⁻¹</td>
<td>30 47.59±4.52</td>
<td>41.14±3.64</td>
</tr>
<tr>
<td>DLNO, ml/min⁻¹·mmHg⁻¹</td>
<td>45 57.53±4.64</td>
<td>50.63±3.89</td>
</tr>
<tr>
<td>DmCO, ml/min⁻¹·mmHg⁻¹</td>
<td>30 24.38±1.37</td>
<td>25.49±2.13</td>
</tr>
<tr>
<td>DmCO, ml/min⁻¹·mmHg⁻¹</td>
<td>45 27.55±1.30</td>
<td>24.97±0.90</td>
</tr>
<tr>
<td>Vc, ml</td>
<td>30 57.9±3.5</td>
<td>41.5±1.18</td>
</tr>
<tr>
<td>Vc, ml</td>
<td>45 58.1±3.8</td>
<td>46.0±1.91</td>
</tr>
</tbody>
</table>

Values are means ± SE. RA, retinoic acid; EELV, end-inspiratory lung volume; DLCO-std, diffusing capacity for carbon monoxide expressed at hemoglobin = 14.6 g/dl and alveolar PO2 = 120 Torr; DLNO, diffusing capacity for nitric oxide; DmCO, membrane diffusing capacity for carbon monoxide; Vc, pulmonary capillary blood volume. *P < 0.05; †P < 0.01; §P < 0.001 vs. placebo.

Fig. 2. Transpulmonary pressure-lung volume relationships were not significantly different between retinoic acid (RA)- and placebo-treated groups. Values are means ± SE. Not significant (NS): P > 0.05 between groups by repeated-measures ANOVA.

Fig. 4. Pulmonary capillary blood volume estimated at rest by the rebreathing technique was significantly lower in RA-treated than in placebo-treated animals. Values are means ± SE.

Drug treatment. One animal receiving RA developed insidious weight loss, skin lesions, anemia, leukocytosis, and elevated liver enzymes. No infectious cause could be found. Chest X-ray was clear. Biopsy of skin lesions showed nonspecific inflammation. This animal did not respond to cessation of RA treatment, empiric antibiotic, or anti-inflammatory treatment and had to be euthanized because of continued clinical deterioration. Results from this animal were not used; therefore, physiological data were available from five RA-treated and six placebo-treated animals. The other animals tolerated drug treatment without complication. Body weight and systemic hemoglobin concentration did not change significantly during follow-up (Table 1); liver enzymes and renal function also remained normal. One RA-treated and one placebo-treated animal died of sudden cardiorespiratory arrest during induction of anesthesia for CT scan. Thus postmortem morphometric data were available from four RA-treated and five placebo-treated animals.

Physiological studies. At a given transpulmonary pressure, lung volume was slightly but not significantly lower in animals receiving RA compared with those receiving placebo (Fig. 2). Lung volume, cardiac output, DLCO-std, DLNO, and DmCO did not differ significantly between groups at an inflation volume of 30 ml/kg above EELV (Table 1). At a higher inflation volume (45 ml/kg above EELV), DLCO-std was significantly lower in RA-treated animals (Table 1 and Fig. 3). In RA-treated animals compared with placebo, Vc was significantly lower at either lung volume (Fig. 4).
Radiological results. Lung air and tissue volumes assessed by CT scan were not different between RA- and placebo-treated groups. Similarly, volume densities of individual cell compartments per unit volume of septum as well as alveolar and capillary surface densities were not different between groups (Table 2). Volume densities of coarse parenchyma, fine parenchyma, and alveolar septal per unit lung volume were not significantly different between groups, whether measured under positive airway pressure or after serial sectioning. Morphometric capillary hematocrit, the arithmetic and harmonic mean septal thickness, and alveolar morphology were also similar (Table 2). Volume densities of coarse parenchyma, fine parenchyma, and alveolar septal per unit lung volume were not significantly different between groups. Similarly, volume densities of individual cell compartments per unit volume of septum as well as alveolar and capillary surface densities were not different between groups (Table 3). As a result, absolute volume and surface area of various cell compartments, as well as morphometric estimates of diffusing capacity components, were also not different between groups (Table 2).

Double-capillary profiles. Following left PNX, the prevalence of double-capillary profiles, expressed as a percentage of total capillary intercepts, is unchanged from that in sham animals and similar in RA- or placebo-treated animals, indicating normal alveolar-capillary morphology. In comparison, the prevalence of double-capillary profile was significantly increased in RA-treated animals following right PNX (P < 0.01 (45)), indicating altered alveolar-capillary morphology, consistent with neocapillary formation or remodeling (Fig. 6).

Regional response. In animals following right PNX, we had observed that RA-induced structural changes were more pronounced in the lower stratum than in the upper stratum (45). In the present animals after left PNX, there was no significant difference in the structural response between upper and lower

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**Table 2. Effect of retinoic acid on the remaining lung following left pneumonectomy**

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Retinoic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>25.3±2.2</td>
<td>26.0±2.1</td>
</tr>
<tr>
<td>Morphometric hematocrit</td>
<td>0.54±0.016</td>
<td>0.518±0.002</td>
</tr>
<tr>
<td>Harmonic mean barrier thickness, μm</td>
<td>0.84±0.03</td>
<td>0.81±0.03</td>
</tr>
<tr>
<td>Arithmetic mean septal thickness, μm</td>
<td>5.98±0.28</td>
<td>5.42±0.38</td>
</tr>
<tr>
<td>Right lung volume, ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immersion method</td>
<td>1.551±182</td>
<td>1.557±124</td>
</tr>
<tr>
<td>Cavalieri method</td>
<td>1.160±87</td>
<td>1.148±57</td>
</tr>
<tr>
<td>Absolute volume in right lung, ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Septum</td>
<td>94.11±13.48</td>
<td>77.1±10.06</td>
</tr>
<tr>
<td>Septal tissue</td>
<td>37.37±5.81</td>
<td>30.54±4.82</td>
</tr>
<tr>
<td>Septal capillary</td>
<td>53.52±9.80</td>
<td>46.55±5.81</td>
</tr>
<tr>
<td>Type I epithelium</td>
<td>6.58±1.19</td>
<td>5.84±1.36</td>
</tr>
<tr>
<td>Type II epithelium</td>
<td>5.81±0.38</td>
<td>4.99±0.51</td>
</tr>
<tr>
<td>Total epithelium</td>
<td>12.39±1.42</td>
<td>10.82±1.60</td>
</tr>
<tr>
<td>Total interstitium</td>
<td>15.38±2.94</td>
<td>12.05±1.79</td>
</tr>
<tr>
<td>Endothelium</td>
<td>9.61±1.53</td>
<td>7.69±1.47</td>
</tr>
<tr>
<td>Absolute surface area in right lung, m²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alveolar surface</td>
<td>32.7±5.8</td>
<td>29.2±4.4</td>
</tr>
<tr>
<td>Capillary surface</td>
<td>34.1±5.4</td>
<td>30.6±4.6</td>
</tr>
<tr>
<td>Morphometric diffusing capacity for oxygen; ml·s⁻¹·mmHg⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythrocyte (Dco₂)</td>
<td>3.460±0.633</td>
<td>3.010±0.376</td>
</tr>
<tr>
<td>Membrane barrier (Dbo₂)</td>
<td>2.171±0.311</td>
<td>2.070±0.332</td>
</tr>
<tr>
<td>Lung (Dco₂)</td>
<td>1.296±0.217</td>
<td>1.198±0.157</td>
</tr>
</tbody>
</table>

Values are means ± SE. Dco₂, erythrocyte diffusing capacity for oxygen; Dbo₂, membrane barrier diffusing capacity for oxygen; Dco₂, lung oxygen diffusing capacity. No significant difference between groups by unpaired t-test (P > 0.05).
strata in either RA or placebo treatment group (data not shown).

DISCUSSION

Summary of results. In the adult dog following 45% lung resection by left PNX, mechanical strain of the remaining lung is insufficient for the reinitiation of cellular growth activities; adaptation occurs exclusively via greater utilization of existing physiological reserves and structural remodeling of the remaining lung without additional growth of new gas-exchange tissue (4, 9). Supplementing the diet with RA following left PNX during the period of most active adaptation does not alter any of the structural indexes of alveolar growth, nor does it improve resting lung function. In fact, RA treatment may be detrimental, as evidenced by a significantly lower resting lung diffusing capacity in RA-treated animals compared with placebo controls.

This response pattern stands in contrast to that following 55% resection by right PNX, where mechanical lung strain exceeds the threshold for reinitiating cellular growth activities. In the remaining lung, new gas-exchange tissue is added to further improve lung function above and beyond that achieved via utilization of reserves and structural remodeling (10, 11). RA supplementation following right PNX selectively enhances compensatory structural response of the alveolar septum and capillary endothelium. Because RA-induced structural enhancement is nonuniform and associated with distortion of septal architecture, there is no net gain in diffusing capacity of the lung or membrane (7, 45). The divergent response patterns following left or right PNX suggest that RA is a promoter of existing alveolar growth but not an initiator of alveolar growth.

Critique of methods. We showed previously that this dose of orally administered all-trans RA accumulates in lung tissue and elicits definite molecular, physiological, and morphological response (45). It is unlikely that a larger dose or longer treatment duration would yield better results, given that the incidence of RA toxicity would certainly increase. The chronic systemic syndrome observed in one of the present animals is compatible with RA toxicity, although this diagnosis remains presumptive. The animals were litter matched to minimize variability. We have previously shown in dogs receiving exogenous RA after right PNX and studied by the same protocol and techniques (45) that four animals per group were sufficient to demonstrate significant differences in key physiological and structural parameters between treatment and placebo groups.

RA enhances active compensatory lung growth. Following right PNX, alveolar cellular growth is reinitiated and involves all septal constituents to eventually restore normal alveolar morphology, even as lung volume nearly doubles. Superimposing RA supplementation on this endogenous response further enhances the increase in alveolar capillary blood volume and growth of alveolar endothelial cells without enhancing growth of the epithelium or interstitium (45). In addition, RA treatment alters capillary morphology, resulting in a significantly higher prevalence of double-capillary profiles typically seen in the immature growing lung (45). These ultrastructural changes distort the three-dimensional septal architecture, leading to an increased septal volume density but a low septal surface-to-volume ratio, i.e., no increase in gas-exchange surface area or in components of lung diffusing capacity estimated by physiological or morphometric methods (7, 45). This pattern of cellular, morphological, and functional dissociation provides a concrete example of “dysanaptic” septal growth, where selective manipulation of an endogenous cellular response leads to distorted structure-function relationships that ultimately limit compensation of the whole organ.

RA has no effect in the absence of active compensatory lung growth. In contrast to the alveolar-capillary growth response and the increase of double-capillary profiles in the remaining lung following right PNX, in the present study after left PNX, adding RA failed to enhance alveolar capillary growth or alter alveolar morphology and, in fact, caused a reduction in resting lung diffusing capacity. These combined studies suggest that RA further amplifies alveolar growth and remodeling only when an endogenous growth stimulus already exists. Our results differ somewhat from that of Kaza et al. (15) in adult rats treated with RA after left PNX. They reported that RA-treated animals showed a higher weight, volume of respiratory air spaces, and cell proliferation index in the remaining lung, although alveolar surface area was not increased and septal ultrastructure was not assessed. The divergent results from rat and dog studies are not incompatible. In rodents, the thorax and lungs continue to grow throughout most of the animal’s life span; i.e., the developmental program of lung growth never completely shuts down. Even moderate additional signals, such as resection of two lobes (3), trigger vigorous growth and remodeling in the remaining lung, and these active processes are susceptible to amplification by RA supplementation. In contrast, in adult large mammals, the developmental program of lung growth becomes quiescent after reaching somatic maturity (~12 mo of age in the dog). Subsequently, a much stronger stimulus, e.g., >45% lung resection, must be instituted to reinitiate active lung growth. The lack of response to supplemental RA in dogs is, therefore, consistent with the subthreshold stimuli and inactive growth pathways following left PNX.

On the other hand, the lack of response to RA supplementation in our dogs after left PNX is consistent with findings by Lucey et al. (20) that showed no effect of RA treatment on air

![Fig. 7. A framework for interpreting the divergent compensatory response patterns to exogenous RA supplementation following R-PNX and L-PNX. See text for discussion.](http://jap.physiology.org/)
space size, alveolar surface area, or mRNA expression of elastin or α1-collagen in adult FVB mice with elastase-induced emphysema. Similarly, Tepper et al. (35) administered RA intraperitoneally for 2 wk in adult rats with elastase-induced emphysema and induced only mild changes in lung volume without any improvement in DlCO. Unlike the PNX model, the emphysema model is marked by a heterogeneous distribution of alveolar-capillary injury and destruction, which may hinder compensatory responses by the remaining normal alveolar units.

The above interpretation also extends published data from our laboratory showing that mechanical forces imposed on the remaining lung constitutes the major in vivo signal for reinitiation of alveolar growth following right PNX. When post-PNX mechanical lung strain is minimized, indexes of structural and functional compensation are reduced by −70% (13, 14, 43). By inference, the primary contribution from nonmechanical signals such as hormones and cytokine growth factors to overall lung growth and compensation would be less, although reciprocal interactions between mechanical and nonmechanical signals may preclude a clear-cut separation of their respective contributions. For example, mechanical stretch activates hypoxia-inducible factor-1α, which is classically thought to respond to a nonmechanical signal (5). Mechanical signals are known to initiate a cascade of molecular and cellular events in the lung, including signal transduction (6, 17), gene expression (30), cell proliferation (6, 18), branching morphogenesis (36), apoptosis (31), DNA and protein turnover (2, 32, 44), induction of cytokine growth factors and hormones (16, 37), as well as altered ion and substrate flux (19). The downstream molecular and biochemical events must be tightly regulated and temporally synchronized to prevent tissue distortion. Supplementing a single growth factor such as RA could modify one or more steps along the cascade but likely could not initiate the cascade in the absence of an upstream or primary mechanical signal (Fig. 7). In addition, selective manipulation of the cascade may cause dysynchrony or mismatch of the steps that ultimately offset the expected benefit.

In conclusion, the negative response to exogenous RA after left PNX in this study provides important insight into the mechanisms of growth regulation in the mature lung. We showed that supplementation of RA, while capable of modulating selected components of active post-PNX lung growth, is unable to initiate a balanced compensatory growth response in the absence of another primary signal, i.e., suprathreshold mechanical lung strain. This limitation is likely not unique for RA, but it may also apply to other pharmaceutical agents that are candidates for use to manipulate compensatory lung growth.

ACKNOWLEDGMENTS

We thank Debbie Tuttle Hogg and Richard Hogg for technical assistance in animal studies, Laurie Task and the staff of the Animal Resource Center for animal care, Drs. Donald Massaro, Gloria Massaro, and David Mangelsdorf for helpful advice regarding retinoic acid administration, and Jean Wang for assistance with tissue processing.

GRANTS

This study was supported by National Heart, Lung, and Blood Institute Grants R01 HL-045716, HL-062873, HL-040070, and HL-054060.

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REFERENCES


