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

Xiao Yan, Dennis J. Bellotto, D. Merrill Dane, R. Geoffrey Elmore, Robert L. Johnson, Jr., Aaron S. Estrera, and Connie C. W. Hsia

Departments of Internal Medicine, Pathology, and Cardiovascular and Thoracic Surgery, University of Texas Southwestern Medical Center, Dallas, Texas

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

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 butter and administered by oral gavage four times daily for 4 mo. All dogs were kept in a constant environment of 50% relative humidity and temperature and were killed by CO2 asphyxiation following anesthesia.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
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/kg/day) (18, 29). Aronex Pharmaceuticals, The Woodlands, TX, now Antigenics). Complete blood count, blood chemistry, and liver enzymes were monitored each week during the first postoperative month and then each month. During the last month of RA administration, resting physiological studies and high-resolution CT scan of the thorax were performed under anesthesia. At the end of treatment, animals were killed, and the lungs were processed for morphometric analysis.

**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 end-expiratory lung volume (EELV). A volume of air (15, 30, 45, and 60 ml/kg) was delivered into the lungs and held for 8 s; from each pressure profile the values at 3 s following delivery were recorded. Transpulmonary pressure was the difference between esophageal and mouth pressures. The inflation volume was incremented and decremented in a stepwise fashion; duplicate measurements were averaged. Pressure-volume curves were analyzed by the model of Salazar and Knowles (29) with the statistical method of Pengelly (25). The derived constants were used to estimate lung volume at predetermined transpulmonary pressures for comparison between groups by repeated-measures ANOVA.

**Rebreathing measurements.** Lung volumes, cardiac output, diffusing capacity for carbon monoxide (DLCO), and nitric oxide (DlNO), and sepatial (tissue plus blood) volume were measured by a rebreathing technique (11). The test gas mixture, consisting of 0.3% carbon monoxide (CO), 0.3% methane, 0.8% acetylene, either in 21% O2 and 0.3% methane, 0.8% acetylene, either in 21% O2 and 7% CO2 for 3 min to ensure equilibration with resident alveolar gas. At a selected end expiration, the expiratory port was occluded. Following lung expansion with three cumulative tidal breaths and 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, Sievers 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 (P2O2), we estimated membrane diffusing capacity for CO (DMCO) and pulmonary capillary blood volume (Vc) using the Roughton-Forster relationship:

\[
\frac{1}{DLCO} = \frac{1}{DMCO} + \frac{1}{\Theta CO \cdot Vc}
\]

where \(\Theta CO\) is the empirical rate of CO uptake by dog whole blood at 37°C (in \(\text{ml CO} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1} \cdot \text{ml blood}^{-1}\)) calculated from the mean \(P_{A,CO}\) (in Torr) during rebreathing and the hemoglobin concentration \([\text{Hb}]\) in g/dl:

\[
\frac{1}{\Theta CO} = (0.929 + 0.00517 \cdot P_{A,CO}) \cdot \frac{14.6}{[\text{Hb}]}
\]

Estimates of DMCO and Vc were then used to calculate the DLCO expected under standardized conditions (DLCO-sta), i.e., at a constant \(P_{A,CO} = 120\) Torr and \([\text{Hb}] = 14.6\) 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 sepatial volume for gas exchange was estimated from the extrapolated intercept of acetylene disappearance to time 0. Estimates of sepatial 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
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 (pentobarbital 25 mg/kg iv), intubated with a cuffed 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 hemidiaphragm to collapse the remaining lung. The remaining lung was reinfated within the thorax by tracheal instillation of 2.5% hemidiaphragm to collapse the remaining lung. The remaining lung was barbital 25 mg/kg iv), intubated with a cuffed endotracheal tube via overnight, premedicated (atropine 0.05 mg/kg sc), anesthetized (pen-

Morphometric lung diffusing capacity. Diffusing capacities for oxygen (DbO2) 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 (DbO2) and within capillary erythrocytes (DeO2):

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

where

\[
D_{bO_2} = K_{bO_2} \left( S_a + S_e \right) \\
D_{eO_2} = \Theta_{eO_2} \cdot V_c
\]

and

\[
\Theta_{eO_2} = \Theta_{eO_2} \cdot V_c
\]

where Sa and Se are the measured alveolar and capillary surface areas, respectively; KbO2 is the Krogh diffusion coefficient for O2 in tissue and plasma \((5.5 \times 10^{-10} \text{ cm}^2 \cdot \text{s}^{-1} \cdot \text{mmHg}^{-1})\) (42); and \(\Theta_{eO_2}\) is the rate of O2 uptake by dog whole blood. We used \(\Theta_{eO_2} = 0.06466 \text{ ml O}_2 \cdot \text{ml blood}^{-1} \cdot \text{s}^{-1} \cdot \text{mmHg}^{-1}\) 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 \(P_{AO_2} = 100 \text{ Torr}\). 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...
adult dogs following right PNX treated with RA or placebo (n = 4 each) (45).

Data analysis. Data are expressed as means ± SE and compared between groups by one-way or repeated-measures ANOVA (Statview 5.0, SAS Institute, Cary, NC). Differences were considered significant if \( P < 0.05 \).

RESULTS

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 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, \( DL_{CO-std} \), \( DL_{NO} \), and \( Dm_{CO} \) 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), \( DL_{CO-std} \) was significantly lower in RA-treated animals compared with placebo, \( Vc \) was significantly lower at either lung volume (Fig. 4).

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</td>
<td>1,744 ± 90</td>
</tr>
<tr>
<td>45</td>
<td>2,221 ± 109</td>
<td>2,165 ± 104</td>
</tr>
<tr>
<td>EELV, ml</td>
<td>30</td>
<td>782 ± 60</td>
</tr>
<tr>
<td>45</td>
<td>817 ± 64</td>
<td>777 ± 38</td>
</tr>
<tr>
<td>Cardiac output, ml/min</td>
<td>30</td>
<td>3,116 ± 225</td>
</tr>
<tr>
<td>45</td>
<td>3,289 ± 214</td>
<td>2,910 ± 112</td>
</tr>
<tr>
<td>Stroke volume, ml</td>
<td>30</td>
<td>20.4 ± 0.8</td>
</tr>
<tr>
<td>45</td>
<td>21.4 ± 0.7</td>
<td>20.5 ± 0.8</td>
</tr>
<tr>
<td>( DL_{CO-std} ), ml/min(^{-1})·mmHg(^{-1} )</td>
<td>30</td>
<td>19.08 ± 1.07</td>
</tr>
<tr>
<td>45</td>
<td>20.81 ± 0.94</td>
<td>18.44 ± 0.67*</td>
</tr>
<tr>
<td>( DL_{NO} ), ml/min(^{-1})·mmHg(^{-1} )</td>
<td>30</td>
<td>47.59 ± 4.52</td>
</tr>
<tr>
<td>45</td>
<td>57.53 ± 4.64</td>
<td>50.63 ± 3.89</td>
</tr>
<tr>
<td>( Dm_{CO} ), ml/min(^{-1})·mmHg(^{-1} )</td>
<td>30</td>
<td>24.38 ± 1.37</td>
</tr>
<tr>
<td>45</td>
<td>27.55 ± 1.30</td>
<td>24.97 ± 0.90</td>
</tr>
<tr>
<td>( Vc ), ml</td>
<td>30</td>
<td>57.9 ± 3.5</td>
</tr>
<tr>
<td>45</td>
<td>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; EILV, end-expiratory lung volume; \( DL_{CO-std} \), diffusing capacity for carbon monoxide expressed at hemoglobin = 14.6 g/dl and alveolar \( PO_2 = 120 \) Torr; \( DL_{NO} \), diffusing capacity for nitric oxide; \( Dm_{CO} \), 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. 3. Lung diffusing capacity for carbon monoxide standardized to a constant hemoglobin concentration and alveolar oxygen tension (\( DL_{CO-std} \)) was not different between treatment groups at a lung volume of 30 ml/kg above end-expiratory lung volume, but it was significantly lower in RA-treated animals at a higher lung volume. Values are means ± SE.

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.
Radiological results. Lung air and tissue volumes assessed by CT scan were not significantly different between RA- and placebo-treated groups. Values are means ± SE.

Morphometric results. After RA treatment compared with placebo, absolute volume of the fixed lung was similar 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, the 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 strata.

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>0.544±0.016</td>
<td>0.518±0.020</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>Immersion method</td>
<td>1,160±87</td>
</tr>
<tr>
<td>Absolute volume in right lung, ml</td>
<td>Septum</td>
<td>94.11±13.48</td>
</tr>
<tr>
<td></td>
<td>Septal tissue</td>
<td>37.37±5.81</td>
</tr>
<tr>
<td></td>
<td>Septal capillary</td>
<td>53.52±9.80</td>
</tr>
<tr>
<td></td>
<td>Type I epithelium</td>
<td>6.58±1.19</td>
</tr>
<tr>
<td></td>
<td>Type II epithelium</td>
<td>5.81±0.38</td>
</tr>
<tr>
<td></td>
<td>Total epithelium</td>
<td>12.39±1.42</td>
</tr>
<tr>
<td></td>
<td>Total interstitium</td>
<td>15.38±2.94</td>
</tr>
<tr>
<td></td>
<td>Endothelium</td>
<td>9.61±1.53</td>
</tr>
<tr>
<td>Absolute surface area in right lung, m²</td>
<td>Alveolar surface</td>
<td>32.7±5.8</td>
</tr>
<tr>
<td></td>
<td>Capillary surface</td>
<td>34.1±5.4</td>
</tr>
<tr>
<td>Morphometric diffusing capacity for oxygen, m²s⁻¹mmHg⁻¹</td>
<td>Erythrocyte (Dco₂)</td>
<td>3.460±0.633</td>
</tr>
<tr>
<td></td>
<td>Membrane barrier (Dco₂)</td>
<td>2.171±0.311</td>
</tr>
<tr>
<td></td>
<td>Lung (Dco₂)</td>
<td>1.296±0.217</td>
</tr>
</tbody>
</table>

Values are means ± SE. No significant difference between groups by unpaired t-test (P > 0.05).

Fig. 5. Air and tissue volumes in the remaining right lung estimated from CT scan were not significantly different between RA- and placebo-treated groups. Values are means ± SE.

Fig. 6. The frequency of double septal capillary profiles, expressed as a percentage of total capillary profiles, was similar in RA- and placebo-treated animals following left PNX (L-PNX RA and L-PNX placebo, respectively) and compared with sham controls. The frequency of double-capillary profiles was significantly higher in RA-treated animals following right PNX (R-PNX RA) compared with placebo (R-PNX placebo) and with sham controls (45). Values are means ± SE. *P < 0.01 vs. all other groups.
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 instigated 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.
space size, alveolar surface area, or mRNA expression of elastin or \( \alpha_1 \)-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 \( D_{LCO} \). 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-1\( \alpha \), 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 pharmacological 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.

DISCLAIMER

The contents of this article are solely the responsibility of the authors and do not necessarily represent the official views of the National Heart, Lung, and Blood Institute or of the National Institutes of Health.

REFERENCES


32. Smith PG, Moreno R, and Ikebe M.