Retinoic acid-induced alveolar cellular growth does not improve function after right pneumonectomy

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Dane, D. Merrill, Xiao Yan, Rahul M. Tamhane, Robert L. Johnson, Jr., Aaron S. Estrera, Deborah C. Hogg, Richard T. Hogg, and Connie C. W. Hsia. Retinoic acid-induced alveolar cellular growth does not improve function after right pneumonectomy. J Appl Physiol 96: 1090–1096, 2004. First published September 23, 2003; 10.1152/japplphysiol.00900.2002.—To determine whether all-trans retinoic acid (RA) treatment enhances lung function during compensatory lung growth in fully mature animals, adult male dogs (n = 4) received 2 mg·kg−1·day−1 po RA 4 days/wk beginning the day after right pneumonectomy (R-PNX, 55–58% resection). Litter-matched male R-PNX controls (n = 4) received placebo. After 3 mo, transpulmonary pressure (TPP)-lung volume relationships, diffusing capacities for carbon monoxide and nitric oxide, cardiac output, and septal volume (Vtiss-RB) were measured under anesthesia by a re-breathing technique at two lung volumes. Lung air and tissue volumes (Vair-CT and Vtiss-CT) were also measured from high-resolution computerized tomographic (CT) scans at a constant TPP. In RA-treated dogs compared with controls, TPP-lung volume relationships were similar. Diffusing capacities for carbon monoxide and nitric oxide were significantly impaired at a lower lung volume but similar at a high lung volume. Whereas Vtiss-RB was significantly lower at both lung volumes in RA-treated animals, Vair-CT and Vtiss-CT were not different between groups; results suggest uneven distribution of ventilation consistent with distortion of alveolar geometry and/or altered small airway function induced by RA. We conclude that RA does not improve resting pulmonary function during the early months after R-PNX despite histological evidence of its action in enhancing alveolar cellular growth in the remaining lung.

compensatory lung growth; dog; carbon monoxide diffusing capacity; nitric oxide diffusing capacity; computerized tomographic scan

Retinoic acid (RA) regulates embryonic branching morphogenesis (23) and the expression of a wide variety of genes and gene products in the lung (8, 9). Exogenous RA administration in rats enhances indexes of alveolar septal formation during postnatal development (26) and after dexamethasone treatment (28), hyperoxic lung injury (49), elastase-induced emphysema (27), and pneumonectomy (PNX) (19). However, altered alveolar morphology attributed to RA is not associated with improved lung function in rats with emphysema (48).

Important species differences in lung structure and function complicate the extrapolation of conclusions from rodents to large mammals. In the rodent, the epiphyses do not close (6), somatic as well as lung growth continues throughout life, and there is no final stable size of the thorax or the lung. In addition, the rodent lung possesses a simpler architecture and limited physiological reserves. These factors may explain the high susceptibility of rodent lungs to a variety of growth-inducing signals. In contrast, in large mammals the rib cage and lungs attain their maximum size on reaching somatic maturity when the epiphyses close. Thereafter, adaptation to destructive lung disease occurs primarily via greater utilization of the remaining alveolar-capillary reserves. Reinitiation of alveolar tissue growth is only possible in the presence of very strong signals and after physiological reserves are exhausted. In adult dogs, major lung resection by PNX reproducibly induces significant although incomplete compensatory growth of the remaining lung but only after a threshold of lung resection (~50%) is exceeded (13, 14). The major signal reinitiating lung growth after PNX is mechanical lung strain (17), although other nonmechanical signals must also play a role. Effective pharmacological stimulation of alveolar septal growth should measurably improve gas-exchange function. Our aim was to determine the in vivo effects of exogenous RA on post-PNX lung growth and function. Our hypotheses were that 1) exogenous RA treatment enhances compensatory alveolar growth in the remaining lung after right PNX and 2) RA-enhanced cellular growth improves lung function. We administered RA or placebo to litter- and sex-matched adult dogs after right PNX (resection of 55–58% of lung tissue). We measured resting lung function by a rebreathing technique and in vivo air and tissue volumes of the remaining lung by high-resolution computerized tomographic (CT) scan, followed by detailed morphometric assessment of lung structure. The present paper details the physiological and radiological results after 3 mo of treatment during the period of most vigorous adaptation. Morphometric analysis showed a preferential induction of alveolar capillary and endothelial cell volumes and is reported in the companion manuscript (50).

METHODS

All procedures had been approved by the Institutional Animal Care and Use Committee. A flow chart of the experimental design is shown in Fig. 1.

Animal procedures. Eight adult litter-matched male foxhounds underwent right PNX (55–58% lung resection) at ~1 yr of age. The animal was fasted overnight; premedicated with buprenorphine, acepromazine, and glycopyrrolate; anesthetized with isoflurane; and intubated with auffed endotracheal tube. Rectal temperature, heart rate, and transcutaneous oxygen saturation were monitored continuously. In a sterile manner, the right lung was exposed via a lateral thoracotomy through the fifth intercostal space. Blood vessels of each lobe were dissected free, tied
with silk ligature, and cut. The right main-stem bronchus was stapled and cut. The bronchial stump was oversewn with loose mediastinal tissue for added protection and then immersed under saline to check for leaks. After hemostasis was ensured, the chest wall was closed in five layers. Topical lidocaine was applied to the intercostal nerve and muscle during closure. Residual air in the right hemithorax was aspirated. Buprenorphine was administered postoperatively at regular intervals for analgesia, and antibiotics were administered for 7 days. Animals were nearly always up and about and eating a regular diet by the next day. Wound dressings were changed daily and skin stitches were removed after 7 to 10 days.

**Drug administration.** Beginning 1 day after PNX, dogs were divided into two litter-matched groups. The RA group \( (n = 4) \) received all-trans RA (Sigma Chemical, St. Louis, MO) at 2 mg/kg po given 4 days/wk. The drug was dissolved in a small amount of alcohol in a light-protected environment as per the manufacturer’s instruction. The dissolved drug was suspended in 1 ml of vegetable oil and mixed thoroughly. The suspension was mixed with 1 tablespoon of honey and 1 tablespoon of peanut butter and immediately given to the dog either in a bowl or fed into its mouth by a spoon. The animal was observed until all the peanut butter had been swallowed. A drug holiday of 3 days/wk was provided to minimize the induction of metabolism. This regimen is kindly suggested by Drs. Donald and Gloria Massaro (Lung Biology Laboratory, Georgetown University). The control littermates \( (n = 4) \) received only the oil diluent in honey and peanut butter (placebo) by the same schedule and delivery method.

Oral all-trans RA is rapidly absorbed. The dose (2 mg/kg⁻¹·day⁻¹) was higher than that previously given intraperitoneally to rats (0.5 mg·kg⁻¹·day⁻¹) (27, 48) and orally to emphysema patients in pilot clinical studies (1 mg·kg⁻¹·day⁻¹) (24) but well below the level known to cause toxicity in dogs (5–10 mg·kg⁻¹·day⁻¹). Aronex Pharmaceuticals, The Woodlands, TX, now Antigenics). Dogs were fed a standard unrestricted diet. After 3 mo of drug administration, resting physiological studies and high-resolution CT scan of the thorax were performed at rest under anesthesia. At the end of 4 mo of treatment, animals were killed and the lungs were processed for morphometric analysis, detailed in the companion manuscript (50) (Fig. 1).

To demonstrate that the drug accumulated in lung tissue and exerted biological action, three additional normal adult dogs received oral all-trans RA by the same schedule for 3, 7, or 90 days. Each animal was killed at the end of the treatment period and the lung tissue was sampled for immunohistochemical localization of all-trans RA and for immunoblot assays to determine the expression of specific cellular markers: surfactant protein-A and pro-surfactant protein-C. Results were qualitatively compared with those in normal untreated dog lung tissue. These procedures are detailed in the companion manuscript (50).

**Resting lung function.** After 3 mo of treatment, lung function was measured in the supine position under anesthesia by a previously described rebreathing method (45). The animal was anesthetized and intubated with a cuffed endotracheal tube and mechanically ventilated (model 607, Harvard Apparatus, Millis, MA) at 15 ml/kg of tidal volume and a rate sufficient to suppress spontaneous ventilation. A latex balloon-tipped catheter was inserted into the distal third of the esophagus and connected to a Validyne pressure transducer, a Harvard-Physiologic, and a chart recorder to measure esophageal pressure changes. An identical apparatus was employed to measure mouth pressure. Transducers were calibrated by use of a manometer. Rectal temperature, heart rate, and transcutaneous oxygen saturation were monitored. The endotracheal tube was connected to a manifold allowing the animals to be connected to either the ventilator or a 3-liter calibrated syringe containing the desired inspiratory gas mixture. At the beginning of each maneuver, the animals were first given three cumulative tidal breaths (total 45 ml/kg) to fully expand the lungs, followed by passive deflation to end-expiratory lung volume (EELV).

**Static pressure-volume relationship.** From EELV, an inflation volume of 15, 30, 45, and 60 ml/kg was delivered into the lungs and held for 8 s. From each pressure-volume curve, the values at 3 s after volume delivery were recorded. Transpulmonary pressure was taken as the difference between esophageal and mouth pressures. The inflation volume was first incremented and then decremented in a stepwise fashion; duplicate measurements at each lung volume were averaged. Pressure-volume curves were analyzed by the exponential model of Salazar and Knowles (42) with the statistical method of Pengelly (37). The derived constants were used to estimate lung volume at prede-termined transpulmonary pressures and were compared between groups by repeated-measures ANOVA.

**Rebreathing measurements.** Lung volumes, cardiac output, diffusion capacity for carbon monoxide (DLCO) and nitric oxide (DLNO), and seagal (tissue plus blood) volume were measured by a modified rebreathing technique (15). The rebreathing test gas mixture, consisting of 0.3% CO, 0.3% methane, 0.6% acetylene, either in 21% O₂ and a balance of N₂, or in 99% O₂, was humidified and drawn into a 6-liter Mylar reservoir bag. Just before each measurement, NO (40 ppm) was added to the reservoir bag and thoroughly mixed by a mechanical pump. The desired volume of test gas mixture (30 or 45 ml/kg plus apparatus dead space) was drawn into the 3-liter calibrated syringe. The dog was first ventilated with the appropriate background gas (21 or 100% O₂) for 3 min to ensure equilibration with resident alveolar gas. At a selected end expiration, the expiratory port was occluded. After lung expansion with three cumulative tidal breaths and passive deflation to EELV, the test mixture was delivered into the lung and rebreathed in and out of the syringe for 16 s at a rate of 30 breaths/min. Gas concentrations were continuously monitored at the mouth by a chemiluminescence analyzer for NO (NOA280, 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 according to manufacturers’ specifications. All signals were digitized by a computer. 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 were calculated from methane dilution and expressed at BTPS conditions. Cardiac output was calculated from the exponential disappearance of end-tidal acetylene with respect to...
methane, corrected for the intercept of CO disappearance (41). D_L CO and D_M CO were calculated from the exponential disappearance of CO and NO, respectively, with respect to methane (40, 47). End-tidal points were selected from the log linear portion of the disappearance curves; the first 3 breaths and those after 12 s were routinely discarded. From D_L CO measured at two levels of alveolar O2 tension (P_A O2), we estimated membrane diffusing capacity for CO (D_M CO) and pulmonary capillary blood volume (V_c) by using the Roughton-Forster relationship

\[ \frac{1}{D_{L CO}} = \frac{1}{D_{M CO}} + \frac{\Theta_{CO}}{V_c} \cdot \Theta_{CO} \cdot V_c \]

(1)

where \( \Theta_{CO} \) is the empirical rate of CO uptake by whole blood at 37°C [in ml CO/(min·Torr·ml blood)⁻¹] calculated from the mean P_A O2 (in Torr) during rebreathing and the hemoglobin (Hb) concentration (in g/dl)

\[ \frac{1}{\Theta_{CO}} = (0.929 + 0.00517 \cdot P_{A O2}) \cdot \frac{14.6}{[Hb]} \]

(2)

Estimates of D_M CO and V_c were then used to calculate the D_L CO expected under standardized conditions (D_L CO-std), i.e., at a constant P_A O2 = 120 Torr and Hb concentration = 14.6 g/dl. Measurement of D_L CO is equally sensitive to the resistances imposed by alveolar membrane and capillary erythrocytes, whereas D_M CO predominantly reflects the resistance imposed by alveolar membrane, as the resistance offered by erythrocytes to NO uptake is small relative to that offered by the membrane (2, 47).

The effective septal volume for gas exchange was estimated from the extrapolated intercept of acetylene disappearance to time zero; this quantity represents the instantaneous acetylene uptake by alveolar septal tissue and capillary blood. 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 general anesthesia with intravenous propofol. The animal was intubated with a cuffed endotracheal tube, placed supine on the CT table, and anesthesia with intravenous propofol. The animal was intubated with a cuffed endotracheal tube, placed supine on the CT table, and anesthesia with intravenous propofol. The animal was intubated with a cuffed endotracheal tube, placed supine on the CT table, and anesthesia with intravenous propofol.

The images were analyzed by use of Object-Image v.1.62 (a public-domain program based on NIH Image by Norbert Vischer, University of Amsterdam, Netherlands). The area occupied by the 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 thresholding. Area of lung in each image was multiplied by the slice thickness to obtain volume; total lung volume was the sum of the volume from individual images. The CT densities (in Hounsfield units) of tracheal air (\( \rho_{air} \)) and muscle (\( \rho_{muscle} \)) were used to partition total lung volume to total air (\( V_{air} \)) and tissue (\( V_{tissue} \)) volume because the average CT density of lung (\( \rho_{lung} \)) is directly proportional to the ratio of tissue and air

\[ V_{tissue} = \frac{V_{total} \cdot (\rho_{lung} - \rho_{air})}{\rho_{muscle} - \rho_{air}} \]

(3)

\[ V_{air} = V_{total} - V_{tissue} \]

(4)

Results were compared between groups by one-way or repeated-measures ANOVA. Differences were considered significant if \( P < 0.05 \).

RESULTS

All animals tolerated right PNX and the subsequent treatment without complication. There was no clinical evidence of RA toxicity. At the time of right PNX, body weights were essentially the same between placebo and RA groups. Animals treated with RA gained weight during the first 2 mo post-PNX and stabilized thereafter (Fig. 2). During the course of the study, the average weight change was +11% in the RA group and <1% in the placebo group. Because body weights were similar between groups at the beginning of the study and diverged later, we chose to report most of our results in absolute values, without normalization by body weight. Blood hemoglobin concentration was not different between groups (Table 1).

Physiological measurements. Lung volumes (Table 1) and the transpulmonary pressure-lung volume relationship (Fig. 3) were similar between groups. D_L CO-std was lower in the RA group compared with the placebo group, but the difference achieved statistical significance only at the lower lung volume (30 ml/kg above EELV) and not at 45 ml/kg above EELV (Fig. 4). Similarly, D_M CO was significantly lower in the RA group at the lower but not at the higher lung volume (Fig. 5). Estimates of V_c were not significantly different between groups (Table 1). Volume of alveolar septa (including tissue and blood) participating in gas exchange, measured from the extrapolated instantaneous acetylene uptake, was markedly lower by 35–42% at both lung volumes in the RA group compared with the placebo group (Fig. 6).

Lung volumes by CT scan. Average air and tissue volumes per kilogram body weight measured at 20 cmH2O transpulmonary pressure in the remaining lung of PNX dogs in either the RA (72.1 and 10.5 ml/kg, respectively) or placebo (74.5 and 12.7 ml/kg, respectively) treatment group were significantly higher above those expected in the left lung of normal adult dogs (40.2 and 6.0 ml/kg, respectively) and in the similar range as that expected in the remaining left lung of dogs that had undergone right PNX as puppies and were studied as adults (70.5 and 10.1 ml/kg, respectively) (46). Thus post-PNX compensatory lung growth has occurred in the present animals, consistent with that observed in previous cohorts studied in our
In adult dogs, RA treatment for 3 mo after right PNX is associated with 1) lower DLNO and DLCO at resting tidal volume, equivalent to results seen during moderate exercise (30 ml/kg above EELV), but unaltered at higher tidal volume, equivalent to results seen during heavy exercise (45 ml/kg above EELV) compared with placebo controls; 2) unchanged anatomic air or tissue volume in the remaining lung assessed from CT scan; and 3) significantly lower effective volume of septal tissue participating in gas exchange assessed from acetylene uptake, whereas capillary blood volume was not different between groups. The combined data suggest that RA treatment during the early period of compensatory lung growth does not enhance gas-exchange efficiency.

**Critique of methods.** We did not follow blood chemistry in this study. In a subsequent cohort of animals given RA by the regimen after left PNX, there was no significant alteration in blood chemistry, liver enzymes, and renal function measured at monthly intervals. The morphometric analysis in these same animals (50) demonstrates a significant biological action of this dose of RA on alveolar tissue. In addition, three separate normal dogs were given RA by the same regimen for 3, 7, and 26 mo after left PNX and were offered no sign of retinoic acid toxicity.

**DISCUSSION**

**Summary of results.** In adult dogs, RA treatment for 3 mo after right PNX is associated with 1) lower DLNO and DLCO at resting tidal volume, equivalent to results seen during moderate exercise (30 ml/kg above EELV), but unaltered at higher tidal volume, equivalent to results seen during heavy exercise (45 ml/kg above EELV) compared with placebo controls; 2) unchanged anatomic air or tissue volume in the remaining lung assessed from CT scan; and 3) significantly lower effective volume of septal tissue participating in gas exchange assessed from acetylene uptake, whereas capillary blood volume was not different between groups. The combined data suggest that RA treatment during the early period of compensatory lung growth does not enhance gas-exchange efficiency.

**Fig. 3.** Static transpulmonary pressure-lung volume relationship. Values are means ± SE. No significant difference between groups by repeated-measures ANOVA.

**Fig. 4.** Lung diffusing capacity for carbon monoxide expressed at a standard alveolar PO2 of 120 Torr and hemoglobin concentration of 14.6 g/dl (DLCO-std) was lower in the RA treatment group at a lower lung volume (P < 0.05) but not different between groups at a higher lung volume. Values are means ± SE. NS, not significant.

### Table 1. *CT scan and rebreathing data*

<table>
<thead>
<tr>
<th></th>
<th>30 ml/kg Inflation Volume</th>
<th>45 ml/kg Inflation Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>RA</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>23.9 ± 0.3</td>
<td>26.8 ± 0.1</td>
</tr>
<tr>
<td>Hemoglobin, g/dl</td>
<td>12.3 ± 0.3</td>
<td>12.7 ± 0.2</td>
</tr>
<tr>
<td><strong>CT scan data</strong></td>
<td></td>
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<tr>
<td>Total lung volume, liters</td>
<td>2.053 ± 0.012</td>
<td>2.247 ± 0.098</td>
</tr>
<tr>
<td>Air volume, liters</td>
<td>1.742 ± 0.010</td>
<td>1.955 ± 0.078</td>
</tr>
<tr>
<td>Tissue volume, liters</td>
<td>0.303 ± 0.013</td>
<td>0.286 ± 0.018</td>
</tr>
<tr>
<td><strong>Rebreathing data</strong></td>
<td></td>
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<tr>
<td>End-expiratory lung volume, liters</td>
<td>0.535 ± 0.034</td>
<td>0.511 ± 0.041</td>
</tr>
<tr>
<td>End-inspiratory lung volume, liters</td>
<td>1.406 ± 0.035</td>
<td>1.486 ± 0.038</td>
</tr>
<tr>
<td>Septal volume, ml</td>
<td>20.1 ± 17</td>
<td>149 ± 11†</td>
</tr>
<tr>
<td>Cardiac output, l/min</td>
<td>2.24 ± 0.10</td>
<td>2.21 ± 0.10</td>
</tr>
<tr>
<td>Stroke volume, ml</td>
<td>15.9 ± 0.7</td>
<td>18.2 ± 1.0</td>
</tr>
<tr>
<td>DLCO-std, ml·min⁻¹·Torr⁻¹</td>
<td>9.96 ± 0.17</td>
<td>9.13 ± 0.23*</td>
</tr>
<tr>
<td>DLNO (ml·min⁻¹·Torr⁻¹)</td>
<td>39.80 ± 1.80</td>
<td>28.20 ± 1.9†</td>
</tr>
<tr>
<td>Capillary blood volume, ml</td>
<td>49.39 ± 7.85</td>
<td>39.04 ± 2.99</td>
</tr>
</tbody>
</table>

Values are means ± SE. Lung diffusing capacity under standardized conditions (DLCO-std) is expressed at alveolar O2 tension of 120 Torr and hemoglobin concentration of 14.6 g/dl. CT, computerized tomography; RA, retinoic acid; DLNO, lung diffusing capacity for nitric oxide. *P < 0.05, †P < 0.01, ‡P < 0.001 between groups at the same volume.

Laboratory. There were no significant differences in the air or tissue volume of the remaining lung between RA and placebo treatment groups (Table 1). Because lung tissue volume measured by CT scan includes volume of the alveolar septa as well as small airways and blood vessels (<1 mm diameter), these estimates were systematically larger than the septal volume estimated by rebreathing.
90 days. RA immunolabeling increased, associated with increased expression of surfactant protein-A in lung tissue (50). Because both groups were fed and housed similarly, the weight gain during the first 2 mo of RA treatment is likely attributable to the drug. Because the groups began the study with similar body weights, we reported the results in absolute values without normalization by body weight, so the progressive weight change in one group does not complicate data interpretation. It seems unlikely that the short-term weight gain in these adult animals reflected further skeletal growth. Normalization by body weight would have further accentuated the intergroup differences in physiological data; hence, our present approach represents a conservative interpretation of the data. We did not study unoperated animals; however, resting lung function in the placebo group is consistent with previously reported results in adult foxhounds after right PNX (15, 45).

**Cellular and molecular effects of RA.** The in vitro action of RA has been extensively studied. RA regulates embryonic branching morphogenesis via the hox genes to enhance proximal airway growth and suppress distal epithelial buds (3, 4). In cell culture, RA stimulates type II pneumocyte proliferation (33), accelerates alveolar epithelial cell maturation and differentiation (4), stimulates surfactant-phosphatidylcholine biosynthesis (8), and protects against hyperoxia-induced cell cycle arrest (34) as well against the antiproliferative action of tumor necrosis factor-α (1). RA modulates gene expression of fibronectin, collagenase, keratins (25), elastin (30, 31), and surfactant-associated proteins (10) in addition to various receptors for hormones and growth factors (21, 22, 33). The action of RA is partly mediated through synergistic interactions with epidermal growth factor receptor (7, 35, 36, 43).

Postnatal in vivo treatment with RA in rats increases the number of alveolar profiles (26). Exogenous all-trans RA given to neonatal rats with glucocorticosteroid-induced inhibition of septation and to adult tight-skin mice with genetic failure of septation enhances alveolar septal formation without increasing alveolar surface area (28). In adult rats with elastase-induced emphysema (27), RA mitigates the pathological increase of alveolar volume and the diminished alveolar number and surface area. RA also enhances epithelial repair and improves survival of neonatal rats after hyperoxic lung injury, leading to increased collagen content in lung tissue but not additional septal formation (38, 49). Thus RA appears to accelerate septal growth after dexamethasone treatment but not after hyperoxic exposure (49), underscoring the different underlying mechanisms of growth arrest.

Signaling pathways of RA have also been studied extensively. Retinoid binding proteins and RA receptor (RAR) isoforms are regulated differentially within alveolar septa during the period of alveolar formation (12). Treatment of newborn rats with a RAR-β agonist impairs septation, whereas RAR-β knockout mice form alveoli at an earlier developmental stage than wild-type mice during but not after the period of septation (29). Thus molecular signaling of lung growth varies with maturation; suppressing RAR-β signaling may rescue failed septation associated with prematurity. On the other hand, transgenic mice bearing RAR-γ deletions exhibit reduced lung elastin content and impaired alveolar formation (30). Thus different RAR isoforms appear to exert counterbalancing influences on alveolar formation.

**Functional effect of RA.** Given the diverse molecular and cellular effects of RA on different compartments of the lung in vitro and in different models of growth arrest in vivo, the important question is whether RA treatment in adult animals would augment global lung function. Relevant information in the literature is limited. One study, by Tepper et al. (48) in rats with elastase-induced emphysema, found that RA enhances indexes of alveolar septation without improving resting lung function assessed from lung volume and single-breath DLCO. In another study by Rosenthal (39), RA administration to adult dogs with papain-induced emphysema results in partial and transient reductions in total lung capacity and in the pathological increase in alveolar diameter estimated by an inhaled aerosol recovery technique. These reports are consistent with our present results.
**Structure-function correlation.** Combined morphometric and physiological data from this study provide unique insight into the determinants of compensatory lung growth, which may explain the confusing results from previous rat studies. Anatomically, exogenous RA treatment is associated with a significantly higher volume density of the septum, septal thickness, alveolar-capillary blood volume, and endothelial cell volume, whereas alveolar-capillary surface area and morphometric estimates of oxygen diffusing capacity were not significantly altered (50). These morphometric estimates are confirmed by physiological measurements of D\textsubscript{LCO} and D\textsubscript{LNO}; i.e., functional response did not mirror cellular response to RA. Selective RA stimulation of endothelial cell growth is associated with a more immature capillary morphology with a lower surface-to-volume ratio of the septum, indicative of loss of surface complexity, and/or altered alveolar geometry. Thus RA treatment causes distortion of alveolar architecture that offsets any physiological benefit that might be expected from enhancement of cellular growth.

In RA-treated adult PNX dogs, resting D\textsubscript{LCO} and D\textsubscript{LNO} are modestly lower at a low lung volume but normal at a high lung volume compared with that in matched controls. D\textsubscript{LCO} is equally sensitive to the resistances imposed by alveolar membrane and capillary erythrocytes, whereas D\textsubscript{LNO} is predominately sensitive to the resistance imposed by alveolar membrane (2, 47). There is a strong correlation between D\textsubscript{LNO} and the D\textsubscript{MCO} calculated from the classic Roughton-Forster relationship at two or more levels of PA\textsubscript{O\textsubscript{2}} (47). Both D\textsubscript{MCO} (32) and D\textsubscript{LNO} (2, 47) increase more with volume expansion than D\textsubscript{LCO}, reflecting the unfolding of alveolar membrane. Thus RA treatment leads to a lower effective surface for diffusion only at lower lung volumes. Given the similar cardiac output, hematocrit, alveolar surface area, and morphometric diffusing capacities between groups (50), the volume-dependent reduction in physiological diffusing capacity is consistent with a less uniform distribution of ventilation at low lung volumes, suggesting altered alveolar geometry and/or altered airway function induced by RA. Given that V\textsubscript{c} was derived from D\textsubscript{LCO} and therefore also susceptible to the variability caused by uneven distribution of ventilation, it was not surprising that physiological estimates of V\textsubscript{c} did not reflect the higher morphometric estimates of V\textsubscript{c} in RA-treated animals.

Septal volume estimated from the extrapolated intercept of acetylene disappearance represents the volume of septal tissue and resident capillary blood that participates in gas exchange, a quantity shown to correlate strongly with anatomic septal tissue volume measured in the fixed lung (18). Compared with placebo, RA treatment caused a ~40% lower acetylene septal volume at both lung volumes, whereas anatomic septal volume was actually higher (50). Like D\textsubscript{LNO}, acetylene septal volume is sensitive to alterations in surface-to-volume ratio of the septum and the distribution of ventilation. In addition to nonuniform alveolar growth within the septum, RA also exaggerated interlobar differences in alveolar structure, which may have contributed to a more heterogeneous regional distribution of ventilation (50). Uneven distribution of ventilation could also imply regional heterogeneity in airway anatomy and/or dynamic smooth muscle function. Post-PNX airway remodeling normally lags behind alveolar growth (5). Initially after PNX, a reduced airway cross-sectional area and elongation of remaining airways markedly elevates airway resistance (Raw). Subsequent airway dilatation gradually reduces Raw. Long-term compensation in Raw is less complete than in gas exchange (44), i.e., nonuniform or “dysanaptic” lung growth. RA regulates mucin gene expression (11) and tracheobronchial epithelial phenotype (20) and thus may alter post-PNX airway remodeling, accentuate airway-parenchyma dissociation, and limit overall functional adaptation. These issues require further investigation.

We conclude that, in adult dogs during the first 3 mo of compensatory lung growth after right PNX, RA treatment does not improve and may impair gas exchange at rest. The lack of functional improvement is associated with a preferential action of RA on endothelial cell growth and particularly in the lower lobe, which distorts alveolar septal architecture and exaggerates interlobar heterogeneity. Structure-function correlation in this model illustrates a general limitation that occurs when only one component of a finely orchestrated multifactorial physiological response is selectively manipulated. Preferential stimulation of one or a few specific cell types (nonuniform alveolar cellular growth) is associated with architectural distortion within a unit as small as the alveolar septum, leading to distorted physiological interactions among acinar components. Effects of such distortion at a macro or tissue level cannot be predicted from the in vitro action of RA at a cellular or molecular level but can profoundly influence the physiological outcome by limiting functional improvement independent of cellular growth.

It should be pointed out that post-PNX compensation progresses through two overlapping phases. The initial phase of active cellular growth is associated with septal thickening and airway lengthening with limited functional compensation (14, 16). In the later phase, septal remodeling restores normal alveolar architecture and is associated with gradual functional enhancement (14). This study was terminated before remodeling was complete, and we did not stress the respiratory system with exercise. It remains possible that functional improvement could become evident after the cessation of treatment if observations were extended beyond 4 mo. Nonetheless, the early functional impairment at low lung volumes will likely impact clinical applications of this treatment in patients with chronic lung disease.

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**REFERENCES**


