Airway-parenchymal interdependence after airway constriction in rat lung explants

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Adler, Andy, Elizabeth A. Cowley, Jason H. T. Bates, and David H. Eidelman. Airway-parenchymal interdependence after airway constriction in rat lung explants. J. Appl. Physiol. 85(1): 231–237, 1998.—The constriction of pulmonary airways is limited by the tethering effect exerted by parenchymal attachments. To characterize this tethering effect at the scale of intraparenchymal airways, we studied the pattern of parenchymal distortion due to bronchoconstriction in a rat lung explant system. First, we measured the elastic modulus under tension for 2% (wt/vol) agarose alone (37.6 ± 1.5 kPa) and for agarose-filled lung (5.7 ± 1.3 kPa). The latter is similar to the elastic modulus of air-filled lung at total lung capacity (4.5–6 kPa) (S. J. Lai-Fook, T. A. Wilson, R. E. Hyatt, and J. R. Rodarte. J. Appl. Physiol. 40: 508–513, 1976), suggesting that explants can be used as a model of lung tissue distortion. Subsequently, confocal microscopic images of fluorescently labeled 0.5-mm-thick explants prepared from agarose-filled rat lungs inflated to total lung capacity (48 ml/kg) were acquired. Images were taken before and after airway constriction was induced by direct application of 10 mM methacholine, and the pattern of parenchymal distortion was measured from the displacement of tissue landmarks identified in each image for 14 explants. The magnitude of the radial component of tissue displacement was calculated as a function of distance from the airway wall and characterized by a parameter, b, describing the rate at which tissue movement decreased with radial distance. The parameter b was 0.994 ± 0.19 (SE), which is close to the prediction of b = 1 of micromechanical modeling (T. A. Wilson. J. Appl. Physiol. 33: 472–478, 1972). There was significant variability in b, however, which was correlated with the fractional reduction in airway diameter (r = 0.496). Additionally, parenchymal distortion showed significant torsion with respect to the radial direction. This torsion was similar in concentric zones around the airway, suggesting that it originates from inhomogeneity in the parenchyma rather than inhomogeneous airway constriction. Our results demonstrate the significance of the nonlinear mechanical properties of alveolar walls and the anisotropy of the parenchyma in determining the nature of airway-parenchymal interdependence.

confocal microscopy; bronchial responsiveness

THE DEGREE TO WHICH AIRWAYS narrow during bronchoconstriction depends on the force produced by the airway smooth muscle and the load against which the smooth muscle must contract. An important component of this load is the tethering effect exerted by the lung parenchyma attached to the airways. This tethering effect, referred to as airway-parenchymal interdependence, has been investigated by measuring the concentration-response curves of lung resistance and elastance to inhaled or intravenous smooth muscle agonists (1, 3, 8). In normal individuals these curves exhibit plateaus at the highest concentrations of agonist, whereas in asthmatic patients the plateaus are absent or greatly elevated (11, 13). The apparent limit to maximal airway narrowing in normal subjects has been proposed to be due in part to airway-parenchymal interdependence, which could be altered in asthmatic patients (7, 8, 11). Investigations of interdependence at the scale of individual intraparenchymal airways have been difficult, so they have been largely restricted to micromechanical modeling (4, 6, 9, 12, 13, 16). Wilson (16) approximated a constricting intraparenchymal airway as a thin-walled elastic tube embedded in linear isotropic spring-network material. Under these conditions the deformation of the parenchyma surrounding a constricting airway is proportional to the inverse of the radial distance, with the assumption that the fixed outer boundary is far away. We were interested in how well this assumption described the distortion of the parenchyma in vitro.

Direct visualization of bronchoconstriction in intraparenchymal airways is possible with use of the explant technique developed by Dandurand et al. (2). Excised lungs are inflated with an agarose-culture medium that gels when cooled, allowing the preparation of thin slices having sufficient mechanical rigidity to avoid collapse. We extend this explant technique by combining it with confocal microscopy to study the pattern of deformation in the parenchyma surrounding constricting airways. Movement within the parenchyma in peribronchial regions was determined by selecting tissue landmarks in the baseline images and identifying their new positions in the contracted images. This allows two questions to be directly investigated: 1) How far away from the constricting airway does interdependence have an effect? 2) Does the parenchyma deform homogeneously, or is there a significant heterogeneous component?

METHODS

Preparation of animals and explants. All procedures were reviewed and approved by a McGill University Animal Ethics Committee. Male Fischer rats (Harlan Sprague Dawley, Indianapolis, IN) weighing 300–350 g were administered a lethal dose of pentobarbital sodium and placed supine in a laminar flow hood, and their necks, thoraxes, and abdomens were soaked with 70% ethanol. They were then intubated via tracheotomy with a length of sterile polyethylene tubing (PE-240, Intramedic, Becton Dickinson, Parsippany, NJ), and the heart and lungs were excised en bloc.

Explants were prepared as previously described (2). Briefly, a 1% agarose solution (type VII low-gelling-temperature agarose, Sigma Chemical, Oakville, ON, Canada) was slowly instilled into the lungs with a syringe until total lung capacity (TLC), i.e., 48 ml/kg, was achieved. The tracheal tube was then clamped, and the inflated lungs were placed at 4°C for 30 min, allowing the liquid agarose solution within the lungs to gel. After the lungs were cooled, the lung tissue was placed...
uptight in a sterile 35-ml syringe, from which the needle end had been cut away, and a 4% agarose solution was poured into this syringe to surround the tissue. The syringe was then placed at 4°C for 30 min to gel the agarose, resulting in a lung-agarose block that was cut into 0.5-mm-thick transverse sections. These explants were then incubated overnight at 37°C.

Measurement of elastic mechanical properties. To evaluate the rigidities of agarose and agarose-filled lung in comparison to air- and saline-filled lung, we measured the elastic (or Young’s) moduli of agarose gels and of agarose-filled lung and compared these values with those published for air-filled lung. Measurements were made using the apparatus shown as a block diagram in Fig. 1. Liquid agarose was prepared as previously described (2), poured into a cylindrical mold (14 mm ID, 50 mm long), and allowed to set. At each end of the agarose cylinder, machined aluminum handles were set; they gripped the gel and allowed connection to a force transducer at one end and a piston at the other. Additionally, two sonomicrometer crystals (model LTZ-2, Transducer Products, Goshen, CT) were set into the gel facing each other and each was ~5 mm from either handle to avoid the contribution of edge effects from the handles. The sonomicrometer (Triton Technology, San Diego, CA) is designed to measure the distance between two crystals in an aqueous medium over short distances (~10 cm) by measuring the transmission time of sound emitted at one crystal and received at the other. The piezoresistive force transducer (model 31, Sensotec, Columbus, OH) measures the tension or compression applied perpendicular to it. The transducer was calibrated with known weights before each measurement.

Once the gel had set, it was placed in a water bath. One handle was fixed to the force transducer; the other was connected to the piston. After calibration of the distance between the sonomicrometer crystals, slow oscillations (with a period of 5–10 s) at ~5% strain were applied to the piston while the applied force and distance between the sonomicrometer crystals were measured. Force and distance data were acquired using a data-acquisition board (model DT2801A, Data Translation, Marlboro, MA) with LABDAT software (RHT Infodat, Montreal, PQ, Canada) at a sampling rate of 100 Hz, after being low-pass filtered with a cutoff frequency of 20 Hz.

The elastic moduli of agarose and agarose-filled lung samples were calculated from the measurement of force and sonomicrometer crystal distance. Strain (ε) is defined as

\[ \varepsilon = \frac{l - l_0}{l_0} - 1 \]

where l is the distance between crystals and \( l_0 \) is the unstressed distance. Values of \( \varepsilon \) were calculated for each data point sampled. Stress (\( \sigma \)) is defined as

\[ \sigma = \frac{F}{A} \]

where F is the applied force measured by the force transducer and A is the unstressed cross-sectional area of the sample. The elastic modulus (E) is defined as

\[ E = \sigma \varepsilon \]

We calculated E as the slope of the linear regression relation between the values of \( \sigma \) and \( \varepsilon \) for each data point. The E for tension (\( E_t \)) and compression (\( E_c \)) were calculated from the corresponding portions of the data.

Elastic modulus of agarose and agarose-filled lung. Eleven samples of 2 and 4% (wt/vol) agarose were used to make 26 measurements of E at 20°C. We also managed to measure one sample of 1% (wt/vol) agarose; however, because 1% agarose gel is extremely fragile, we were unable to successfully measure more samples, despite repeated trials. The average ratio \( \frac{E_t}{E_c} \) was 1.11 ± 0.07 (SE). The following values for \( E_t \) were measured for agarose gel at 20°C: 160.1 ± 10.7 (SE), 37.6 ± 1.5, and 15.1 kPa for 4, 2, and 1% agarose, respectively.

We employed the same protocol used for the preparation of explants to measure the modulus of agarose-filled lung by filling the lung to TLC (48 ml/kg) with 2% (wt/vol) agarose at 37°C. After the preparation was cooled, a 14-mm-diameter 30- to 50-mm-long cylindrical section was cut from a lobe and inserted into the mold, the sonomicrometer crystals were inserted into a small slice in the lobe, and nylon handles were glued onto the sample with use of Loctite glue (Loctite, Mississauga, ON, Canada). The sample was then placed in the apparatus of Fig. 1, and the protocol described above was applied. Three samples of 2% agarose-filled lung were measured at 22°C, giving an average \( E_t \) of 5.7 ± 1.3 (SE) kPa. This value is similar to the E for whole lung at TLC, suggesting that explants can be used as a model of tissue distortion in lung parenchyma.

Confocal microscopic imaging of explants. Having verified that explants and lung tissue have similar rigidities, we investigated the distortion in the parenchyma surrounding constricting airways by studying confocal microscope images of the explants before and after constriction. To load explants that contained a cross-sectional airway with the fluorescently labeled lectin FITC-wheat germ agglutinin (Molecular Probes, Eugene, OR), we placed them in a 10 µM solution at 37°C for 15 min. They were then examined using an inverted microscope (model IMT-2, Olympus, Tokyo, Japan) with a laser scanning confocal microscope attachment (Insight Plus, Meridian, Okemos, MI). Gray-scale images were recorded using a digital camera (model TM 9701, Pulnix, Sunnyvale, CA), which allowed long exposure times to compensate for the low light levels inherent in confocal microscopy. A baseline image was taken of an ~0.5-mm-diameter airway at ×40 magnification, and then 10 mM methacholine (Sigma Chemical) was directly applied to the explant to induce bronchoconstriction. This dose is known to cause maximal shortening of smooth muscle in this preparation. A second image, referred to as the contracted image, was recorded a minimum of 15 s after the methacholine administration, with care taken to ensure that the bronchoconstriction had reached its full extent.

Fourteen explants, which met the following inclusion criteria, were studied. 1) Total contraction was >10% of the initial airway diameter [defined using the change in airway radius (\( \Delta r_w \)); see below]. 2) The ratio of maximum to minimum diameter of the airway was <2, indicating that the airway...
was roughly circular and had not been cut at an angle. 3) Only one airway was in the image. The properties of the included explants were as follows (means ± SD): 0.720 ± 0.148 mm diameter, 32.1 ± 18.9% change in airway diameter due to constriction, and 1.62 ± 0.23 maximum-to-minimum diameter ratio.

Selection of tissue landmarks and calculation of parenchymal distortion. To study the distribution of tissue movement throughout the image, the movement of tissue landmarks between the baseline and contracted images was determined. A minimum of 100 landmarks per explant were selected on the baseline image by an operator using custom-made software. Landmarks were chosen at sites in the control image, such as intersections of alveolar walls, which could be easily identified in the contracted image.

The position of each landmark was defined by four quantities: the horizontal and vertical coordinates (x₀ and y₀) in the baseline image and the corresponding coordinates in the contracted image (x₁ and y₁). The lumen of the airway in the baseline image was defined by selecting a minimum of 10 points on the epithelial surface and interpolating between them by use of a Fourier series expansion of the points in terms of polar coordinates about their centroid. The radial distance (rᵥ) of a landmark from the airway was taken between (x₀, y₀) and the closest point (xₐw, yₐw) on the airway lumen curve; thus

\[ rᵥ = (x₀ - xₐw, y₀ - yₐw) \] (4)

The rₐw was calculated as the mean distance between the airway lumen curve and its centroid, and Δrₐw was the change in rₐw from baseline to contracted image. The movement vector (mᵥ) for each landmark was

\[ mᵥ = (x₁ - x₀ - Δxₐw - Δxₐwc, y₁ - y₀ - Δyₐw - Δyₐwc) \] (5)

where (x₁ - x₀, y₁ - y₀) is the component due to the difference in the landmark position between the contracted and baseline images, (Δxₐw, Δyₐw) is the bulk movement of the tissue, and (Δxₐwc, Δyₐwc) is the movement of the airway centroid. The bulk tissue movement was defined as the average movement of all landmarks that had rᵥ greater than twice rₐw. The airway centroid movement was defined as the difference in the position of the centroid between the contracted and baseline images.

For each landmark, the radial movement (mᵥ) was calculated by taking the component of movement (mᵥ) in the direction of the radial vector (rᵥ). The torsion angle (θ) was the angle between mᵥ and rᵥ. Positive values of rᵥ indicate rᵥ away from the airway center. The advantage of Eq. 6 is that it separates the magnitude of movement (a) from the rate of decrease of movement with distance (b). Additionally, when b = 1.0, it is equivalent to the result from Wilson for an infinite spring-network model. The parameter b > 1 indicates a more rapid falloff with distance; a lower b indicates a falloff less rapid than Wilson's predictions. SDs for a and b were individually calculated at the optimal value for the other parameter.

RESULTS

Figure 3 shows representative images of unconstricted airways and surrounding parenchyma. These images are representative of two patterns of constriction. In some airways, greater movement was noted near the airway than further into the parenchyma (Fig. 3A); in others, displacements of the surrounding tissue were distributed more evenly through the surrounding structures (Fig. 3B). These differences are reflected in the parameters describing the relationship between the magnitude of radial movement in surrounding tissues (a), its falloff with distance (b), and the fractional change in airway radius (Δrₐw/Δrₐw). In Fig. 3A, a = 0.498, b = 1.263, and Δrₐw/Δrₐw = 0.5165. In Fig. 3B, a = 0.236, b = 0.254; and Δrₐw/Δrₐw = 0.273.

Characterization of interdependence. The range over which interdependence forces caused tissue distortion was determined from the radial movement of landmarks as a function of the distance from the airway.
Characterization of inhomogeneity. The inhomogeneity in the parenchymal movement was determined from the landmark \( u \). Figure 5 shows a histogram of the \( u \) values for the samples shown in Fig. 3. Both samples show a strong central peak near 0° with a distribution on each side. We described this distribution by calculating \( \bar{u} \) and DW, the standard deviation of the \( u \) values. To determine whether the torsion in the parenchymal movement is more pronounced close to the airway wall or further from the airway, landmarks were divided into three zones: 1) landmarks with \( r_v \leq r_{aw} \), 2) those with \( r_{aw} < r_v \leq 2r_{aw} \), and 3) those with \( r_v > 2r_{aw} \). Values of \( \bar{u} \) and DW calculated in each zone and for all zones together are shown in Table 1. The values of \( \bar{u} \) and DW were not significantly different between zones (t-test, \( P > 0.10 \)). There was no significant correlation between \( b \) and DW for all samples (\( r = 0.21, P > 0.20 \)).

Figure 6 plots the value of the parameter \( b \) (in Eq. 6) as a function of \( \Delta r_{aw}/r_{aw} \). There is a significant correlation between \( b \) and \( \Delta r_{aw}/r_{aw} \) (\( r = 0.496, P < 0.05 \)).

The images of Fig. 3 show that regions of parenchyma have areas where all landmarks have similar \( u \). This localized bulk movement is not reflected in the DW, which represents the overall distribution of \( u \). We therefore determined the similarity of each \( u \) to the mean \( u \) of nearby landmarks (\( u_{local} \)). For each landmark, a neighborhood of landmarks with initial positions closer than \( r_{aw} \) to its own position was chosen. The \( u \) values of all landmarks in the neighborhood were then averaged, and the mean difference, \( \Delta u_{local} \), was calculated between each \( u \) and its corresponding \( u_{local} \). DW\(_{local}\) represents the standard deviation of \( u - u_{local} \) values. The average number of landmarks per neighborhood for all samples was 6.3. The average \( \pm SE \Delta u_{avg, local} \) was 0.6 ± 0.2° and DW\(_{local}\) was 36.5 ± 3.4°.

**DISCUSSION**

Airway-parenchymal interdependence is the load exerted by the parenchyma on constricting airways and...
is understood to help protect against airway closure due to smooth muscle agonist challenge. Reduced interdependence has been proposed as a mechanism accounting for bronchial hyperresponsiveness in asthma (8). Unfortunately, the effect of interdependence at the scale of individual intraparenchymal airways has been difficult to study. Wilson (16) proposed a micromechanical model of the distortion of the parenchyma surrounding constricting airways that predicts an inverse correlation between distortion and radial distance. We have verified this hypothesis by measuring the distortion of the parenchyma surrounding individual airways due to smooth muscle contraction after direct application of methacholine to explanted intraparenchymal airways. Confocal microscopic images were taken before and after airway constriction, after which the parenchymal distortion was determined from the movement of landmarks from baseline to constricted image. This model allows two questions to be directly investigated: 1) How far from constricting airways does interdependence have an effect? 2) To what extent is interdependence heterogeneous?

Before addressing these issues, we sought to confirm the suitability of the explant system for this purpose. Because the infusion of agarose into the alveoli mechanically stabilizes lung slices, it is natural to question whether the predominant force-bearing element in the lung explant is the lung tissue or the agarose gel. We measured the E of agarose gel and agarose-filled lung and found that, at least for small distortions, rigidity of explants is similar to that of air-filled lung. Lai-Fook et al. (5) showed the E of dog lungs to be 1.5–2 times the transpulmonary pressure (Ptp), and from the data of Stamenovic and Yager (14) in rabbit lungs we calculated the E to be ~1.5 Ptp. If it is assumed that TLC corresponds to a Ptp of 3 kPa, these data indicate that the E of air-filled lung is 4.5–6 kPa, which is close to our estimate of the E of agarose-filled lung, 5.7 kPa, but significantly less than that of agarose gel for all concentrations tested. Thus, although bulk agarose gel is quite stiff, the mechanical properties of the agarose-lung matrix more closely resemble those of the lung itself than those of the agarose gel. This may be because the alveolar walls separate the agarose into compartments and prevent the gel from directly bearing the

**Table 1.** Average absolute value of $\theta$ and DW of torsion angles in different zones

|        | $|\theta|$ | DW       |
|--------|------------|----------|
| All zones | 11.3 ± 1.7 | 58.3 ± 4.8 |
| Zone 1  | 13.1 ± 2.4 | 59.0 ± 4.3 |
| Zone 2  | 13.2 ± 3.2 | 52.0 ± 3.4 |
| Zone 3  | 19.8 ± 3.3 | 57.6 ± 6.3 |

Values are averages ± SE expressed in degrees. $|\theta|$, Absolute value of torsion angle; DW, distribution width. Zones are defined in terms of radial distance from airway wall: $r_v < r_{aw}$ (zone 1), $r_{aw} < r_v < 2r_{aw}$ (zone 2), and $r_v > 2r_{aw}$ (zone 3), where $r_v$ is radial vector and $r_{aw}$ is airway radius.
force. The similarity of E of the agarose-lung matrix to that of whole lung suggests that explants form a suitable system in which to study parenchymal distortion due to airway constriction.

How far from constricting airways does interdependence have an effect? There was a clear fall off in parenchymal distortion with increasing distance from the airway-epithelial surface, as can be seen in Fig. 3. The rate of decrease of radial movement with distance from the airway-epithelial wall is shown in Fig. 4, whereas the structure of this relationship is described by the parameters a and b of Eq. 6. The parameter a indicates the ratio of landmark movement at the airway-epithelial wall to $\Delta r_{aw}$. Because $\Delta r_{aw}$ is defined as the average movement of the epithelial wall, a is expected to be 1, which is close to its measured value, 1.022 ± 0.07.

The parameter b is a measure of how far from the airway wall interdependence exerts an effect. To the extent that the pattern of parenchymal distortion represents the underlying forces, its value can be considered as an index of the magnitude of interdependence. A large value of b indicates a rapid fall off in parenchymal distortion and thus low interdependence, whereas a low value indicates that movement extends further into the parenchyma and that interdependence is correspondingly higher. The observed average b of 0.994 ± 0.20 matches the continuum linear spring model of Wilson (16), which, under the assumption that the fixed outer boundary was far away, predicts a radial displacement proportional to the inverse of radial distance, which is equivalent to $b = 1$. The assumption of a fixed outer boundary is reasonable in this preparation, because the diameter of the explant was much larger than the airway and there was no mechanism to restrain the movement.

To what extent is interdependence heterogeneous? Although, on average, b was close to 1, there was considerable variability in its value among samples, which indicates that there is heterogeneity in interdependence among airways. This is perhaps not surprising, inasmuch as heterogeneity of interdependence in vivo has also been shown on an acinar level by Mishima et al. (10). This suggests that mechanical models that describe lung tissue as a uniform medium (4, 9, 12, 13, 16) may be inadequate. The value of b was significantly correlated with the degree of airway constriction, as shown in Fig. 6, and the direction of the correlation is consistent with the understanding of interdependence as a load on airway constriction. As b increases, interdependence decreases, with a consequent increase in constriction. Interestingly, there seems to be a clear division of samples into two groups: 1) a group with a high degree of constriction and high b, corresponding to a low degree of interdependence; and 2) a group with less constriction, in which b is low, with a high degree of interdependence. Figure 3, A and B, is representative of the high- and low-constriction groups, respectively. These findings indicate that the degree of coupling between airways and surrounding tissues is heterogeneous and raise the possibility that, in disease states such as asthma, there could be an increased number of such airways that would be predisposed to close more easily.

An additional feature in our data was the pattern of torsion of the parenchymal movement around the airway. For example, in Fig. 3A, there are bulk areas of parenchyma that move at right angles to the direction of airway constriction. These areas of bulk movement often "funnel" toward zones of peribronchial connective tissue, which typically move more directly toward the airway. Additionally, in many samples the airway smooth muscle did not constrict uniformly, which can again be seen in Fig. 3A. Thus heterogeneity in interdependence was present not only among different samples but also within the pattern of constriction for a single airway. It is unknown whether the same pattern of bulk movement would occur in very small peripheral airways with relatively less peribronchial connective tissue.

The distribution of torsion in the parenchymal movement was described by the parameter DW, which remained roughly constant with distance from the airway-epithelial surface. This suggests that torsion in the parenchyma was due primarily to heterogeneity in the parenchyma rather than in the smooth muscle contraction, since heterogeneity in muscle constriction would tend to result in a larger DW in zones close to the airway. We hypothesize that the parenchymal torsion is due to the relative differences in stiffness between peribronchial connective tissue and parenchyma. Interdependence forces would tend to be transmitted by the stiffest tissue, and the distortion of more elastic regions would tend to be oriented toward nearby stiffer tissue. However, because we had access only to parenchymal movements and not to the interdependence forces themselves, we are not able to directly test this hypothesis. Mead et al. (9) argued that interdependence reduces the inhomogeneity of parenchymal deformation. This suggests that $\psi$ in a localized area of tissue should be more uniform than in the parenchyma as a whole. Our finding that the value of DWlocal is 63% of the DW for all zones is consistent with this notion. Also, Balassy et al. (1) found that the variability in changes of regional lung impedance in dogs during induced
constriction was radically increased as lung volume was decreased, presumably also because of reduced interdependence forces.

In summary, we used a rat lung explant system to study parenchymal distortion due to interdependence forces at the scale of intraparenchymal airways. We developed a parameter, $b$, to characterize the extent of interdependence in the parenchymal deformation. Although, on average, $b$ was $-1$, in agreement with previous theoretical studies, there was considerable heterogeneity in interdependence, both among explants and within individual samples. Explants with greater interdependence showed less constriction and vice versa. Tissue movement exhibited significant torsion with respect to the radial direction because of inhomogeneity in the parenchyma rather than inhomogeneity in smooth muscle constriction. Thus, on average, our results agree with the linear continuum micromechanical analysis of Wilson (16). Additionally, however, the data show a variability of interdependence both among samples and in the torsional component of parenchymal distortion. This suggests that the linear analysis of airway-parenchymal interdependence must be extended to account for both the nonlinear stress-strain properties of alveolar walls (15) and the parenchymal anisotropy that exists at the level of the intraparenchymal airway.

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