On the mechanism of mucosal folding in normal and asthmatic airways

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IN ASTHMA, many of the clinical signs and symptoms are due to airway obstruction resulting from smooth muscle constriction. The magnitude of the obstructive response observed for a given degree of smooth muscle activation reflects the contractile capacity of the airway smooth muscle and the resistance to airway deformation due to the structural components of the parenchyma and airway wall. Despite the central role of airway smooth muscle, little is known about airway wall mechanics, especially in chronic asthma where there is significant remodeling of the airway wall. Indeed, chronic remodeling of the airways, characterized by thickening of all regions of the wall (12, 14) and, in particular, the subepithelial collagen layer (25) and smooth muscle hyperplasia (9), has been linked to airway hyperresponsiveness that is a critical phenotypic characteristic of asthma.

Recent studies (18) have recognized a potentially important consequence of airway smooth muscle constriction on airway wall geometry in asthma; i.e., that the luminal boundary folds or buckles as the smooth muscle contracts. Such buckling has also been observed in other biological vessels such as arteries, blood vessels in the myocardium, the eustachian tube, and the gastrointestinal tract. Mucosal folding as a consequence of airway smooth muscle constriction has been observed for many years, accompanied by the suggestion that deeper folds are found in asthmatic airways than in comparably sized normal ones (10). However, to date, no systematic study has been performed that quantifies differences in the number of folds between comparably sized normal and asthmatic airways. We have chosen to investigate the potential effect of the physical features of airway wall structure that could determine the type of airway mucosal folding. As shown in Fig. 1, if numerous folds occur following smooth muscle shortening and if these mucosal folds extend into the luminal space until epithelial cells touch, then a certain degree of luminal narrowing will result. In contrast, in structures with fewer folds, the extent of luminal narrowing when epithelial edges touch is far greater compared with the situation with many folds. These concepts of low- vs. high-frequency folding have been examined by Lambert (17), and it has been demonstrated that a pronounced difference in mucosal folding patterns can have a dramatic effect on airway narrowing. Although the buckling pattern in itself is, therefore, of critical importance in understanding the amount of luminal obstruction for a given contractile stimulus, the physical basis for this distinct mechanical response has not yet been studied.

The previous works of Moreno et al. (20), Lambert et al. (18), and Wiggs et al. (30) were largely based on geometrical arguments concerning the thicker airway wall in asthma, relative to the control airways studied. Lambert et al. (18) studied the importance of mucosal buckling, but the folds were imposed as geometrical constraints. We present in this manuscript a new theory to account for the mechanism of mucosal folding that occurs after smooth muscle shortening. The results offer an explanation for differences in airway narrowing between asthmatic and control airways based on the solid mechanics of the airway wall structure.

MODEL

We chose to model a noncartilaginous conducting airway segment as a bilayered cylindrical structure. This model, shown in cross section in Fig. 2, considers only the airway wall tissue internal to the smooth muscle layer. In the nomenclature introduced by Bai et al. (2), the two depicted regions correspond to some portion of the submucosal region (all loose connective tissue on the luminal side of the smooth muscle) and the mucosal region (which includes the lamina propria or subepithelial collagen layer, the basement mem-
brane, and the epithelium). The “inner” or lumen-bordering layer in our model is taken to be considerably thinner and stiffer than the less organized extracellular matrix between the smooth muscle and this layer. Both layers are assumed to be isotropic linear elastic (Hookean) materials, described by Young's elastic modulus ($E$) and the Poisson ratio ($\nu$). For simplicity, we have further assumed that $\nu \approx 0.5$, thus the materials are incompressible (i.e., volume preserving). In the axial direction, we assumed plane strain, implying no change in longitudinal length during smooth muscle constriction.

To simulate the effect of smooth muscle shortening, we surrounded the outer perimeter of the model with a thin uniform band that constricts, exerting a stress on the outer surface of the thicker layer. The effect of airway wall remodeling as seen in asthma was investigated by thickening both the thicker wall layer internal to the smooth muscle (30) and the thin inner layer (25). In the absence of any experimental data, we also systematically altered the relative intrinsic stiffnesses of the two layers.

This model is completely described by five parameters: the radius to the outside of the inner layer ($R$), the inner and outer layer thicknesses ($t_i$ and $t_o$, respectively), and the elastic moduli of the two layers ($E_i$ and $E_o$). It is convenient to think of the model in terms of three dimensionless ratios: outer thickness ratio ($t_o/R$), inner thickness ratio ($t_i/R$), and stiffness ratio ($E_i/E_o$).

The model, although simple, provides a basic framework that contains the fundamental features of an airway wall internal to the smooth muscle. The model is intended to help understand a mechanism of action, not to necessarily provide an exact numerical prediction of airway behavior.

**METHODS**

The buckling of cylindrical shells under general loading conditions is a well-studied problem (27) as is the analysis of laminated planar plates (1). Unfortunately, the combination of these, a thick-walled laminated cylinder with a stiff inner layer, has not been previously studied. Furthermore, while simpler problems can be addressed by analytical means, the geometrical complexity associated with buckling of a laminated cylinder suggests numerical solution by finite-element analysis. This method, used widely in structural engineering, allows for the mathematical modeling of a complex structure by a collection of many “finite-elements,” within each of which is assumed a simple deformation. Because the validation of both the numerical algorithms and numerical solution by nonlinear finite-element analysis is itself an extremely complex task, we chose to use commercially available software (ABAQUS, version 5.2, HKS, Pawtucket, RI) rather than attempt to generate our own code.

We systematically varied each of the nondimensional parameters ($t_o/R$, $t_i/R$, and $E_i/E_o$) and computed the buckling mode or shape preferred by the model while the other two parameters were held constant. The preferred mode is the one that requires the least strain energy (or the minimum external stress) to induce. First, a linearized buckling analy-
sis (4) was used to rank all possible buckling modes according to the amount of strain energy required to achieve them and identify the one most likely to occur. After the preferred buckling mode was determined for a perfectly cylindrical tube, minute imperfections were introduced into the geometry of the model structure, and a nonlinear static analysis was used to generate quantitative relationships between the external stress applied and the associated internal deformations and stress distributions for an imperfect structure. Imperfections corresponding to a 0.01% shift in geometrical shape from a perfect cylinder were either random, possessing a wide range of possible wavelengths, or specific to the mode predicted by the linearized buckling analysis; both produced similar results. The results presented here were obtained by using the latter method.

To ensure that the size of the individual computational elements is sufficiently small to adequately resolve the solution, we systematically increased the element density until we achieved a consistent buckling pattern. Higher resolution calculations were also performed on a single fold to more accurately assess the internal stress distributions. The unit of pressure used in these results is the pascal; 100 Pa is −1 cmH2O.

RESULTS

Figure 3 shows the results from the linearized buckling analysis as each of the nondimensional parameters was systematically varied while the other two were held constant. The three panels show representative plots where the nonvarying parameters were given the values: \( t_o/R = 0.02 \), \( t_i/R = 0.5 \), and \( E_i/E_o = 10 \). Figure 3A shows that, as the outer structure becomes thicker, little change is seen in the preferred buckling mode. Figure 3B clearly demonstrates that, as the inner region of our model thickens, the number of folds in the preferred buckling mode markedly decreases. Finally, in Fig. 3C, we see that the effect of stiffening the inner layer relative to the outer layer (increasing the \( E_i/E_o \) ratio) has an intermediate effect on the buckling mode.

An example from the nonlinear static analysis is seen in Fig. 4. In this plot, the applied effective external pressure on the outer edge has been normalized by \( E_i^{1/3} E_o^{2/3} \), a form suggested by the buckling behavior of laminated planar composites (1). Figure 4 shows that the change in luminal area as the external pressure is increased initially follows that of an axisymmetric compression, followed by an abrupt change in the character of the solution due to buckling. After buckling, the structure becomes markedly more compliant.

A more detailed view of the pressure stresses within the buckled structure is shown in Fig. 5 and corresponds to the point of maximal area reduction seen in Fig. 4. Figure 5 shows high- and low-pressure stresses (the mean of the radial and circumferential normal stresses) within the structure after buckling. Lower pressures (cold or blue tones) are found within the tissue regions that are folded into the luminal space. Higher pressures (hot or red tones) are found in the adjacent areas that are under compression. Also seen in Fig. 5 is the high-pressure stress and abrupt change in pressure stress between the thin inner region and the thick outer region. The inset of Fig. 5 displays a blow up of a single fold.

Fig. 3. Results of finite-element linearized buckling analyses of 2-layer model, showing how the expected number of folds (N) varies as each of the 3 model parameters is perturbed from an arbitrary reference case while the other 2 are held constant. A: effect of varying outer thickness ratio (t_o/R). B: effect of inner thickness ratio (t_i/R). C: effect of stiffness ratio (E_i/E_o). Reference case: t_o/R = 0.5; t_i/R = 0.02, E_i/E_o = 10.

DISCUSSION

The magnitude of the obstructive response observed in asthma for a given contractile stimulus reflects the capacity of airway smooth muscle and the load against which it acts, i.e., the structural components of the
parenchyma and airway wall. Despite its critical role in this process, little is known about airway wall mechanics, especially in chronic asthma in which there is significant remodeling of the wall (14, 25). This remodeling no doubt contributes to the distinctive change in the pattern of buckling observed in airways, which is now believed to contribute to the asthmatic response. The two-layer composite structure presented here predicts that the inner layer dominates over other modeled characteristics of the wall in determining the number of folds resulting from smooth muscle shortening. Thus we would expect a reduction in the observed number of mucosal folds in a thickened or stiffened inner layer.

Although idealized, this model provides insight into the effect of varying the geometry and intrinsic material stiffnesses of an airway (or other biological vessel) on the resulting folding pattern seen as a result of smooth muscle constriction. The dominant influence is due to the thickness of the stiff subepithelial collagen layer, which has been shown to thicken in asthmatics. This thickening results in a folding pattern with fewer circumferential folds and, consequently, a greater tendency to produce airflow obstruction. Whereas there are many refinements that could be introduced, those investigated to date do not alter the general character of these results.

The thickening of the epithelial basal lamina has been considered a dominant feature of asthma (6). Jeffery et al. (13) have made detailed measurements of the subepithelial collagen layer and have noted a markedly thickened reticular lamina. Roberts (24) commented that this distinct layer is doubled in thickness from ~7 to 14 µm in asthmatic subjects relative to nonasthmatic controls and that this thickened layer is composed of mainly types III and V collagen. It has been suggested that this newly deposited collagen derives from myofibroblasts that populate this layer in asthmatics (5). Roche et al. (25) have commented that the collagen fibers in this subepithelial region appear more densely packed than normal, and Roberts (24) has hypothesized that this would lead to a more mechanically stiff layer relative to the surrounding extracellular matrix. These findings lead us to propose that our theoretical stiff inner layer represents the airway wall tissue consisting of the subepithelial collagen layer and all structures toward the luminal edge.

With these data alone, however, one cannot accurately estimate the amount of stress the smooth muscle must apply to produce significant luminal obstruction; this requires additional information on the airway wall compressive modulus. Preliminary measurements of the tensile stiffness of airways yield a modulus of ~1 kPa (23); with this value, and with the dimensions of the airway represented in Fig. 4, it would require 0.6 kPa of external stress to produce a 50% reduction in cross-sectional area. This estimate can be compared with the stress-generating capacity of airway smooth muscle. Under maximal stimulation, small bronchi are capable of generating pressures as high as 4 kPa under isovolumetric constriction (8). These stress levels can also be compared with the external tethering force of the surrounding lung parenchyma which, for normal lung volumes, would be in the range of ~0.5 kPa. Similarly, the reduction in pressure acting at the inner luminal surface due to the effects of surface tension is only 0.1 kPa for an airway 250 µm in diameter, coated with a liquid having an interfacial tension of 0.025 N/m. The stress estimates are also affected by our choice of linear material properties. Most biological materials exhibit nonlinear stiffening as they are compressed, and this would lead to larger stress values than we have presented.

Although it has not yet been demonstrated for airway wall, stress-induced remodeling is a common trait found in other connective tissues and might well be responsible for the changes that are observed in asthma. Stress levels in the vicinity of the lamina propria and epithelium are quite high (see Fig. 5), easily attaining values of 3 kPa, >10 times as large as the pressure applied by the smooth muscle. Shear stresses and gradients in stress are also high, especially in the vicinity of the stiff inner layer. By comparison, shear stresses due to blood flow as low as 1.5 Pa are known to elicit a variety of biological responses from arterial endothelial cells. If these elevated stresses stimulated production of collagen, elastin, or proteoglycans, the result would be a reduction in the number of folds and a coincident reduction in peak stress levels for a given level of airway constriction. Whereas this remodeling might seem beneficial because there is stress reduction locally and on individual cells, it would, at the same time, allow much more airway occlusion before the folds close up on themselves and resist continued deformation (Fig. 1). Asthmatic thickening of the lamina propria might thus predispose the airway to greater luminal compromise.

These results also have implications for the movement of liquid between the various wall compartments.
and between the wall, the lumen, the lymphatics, and the vascular space. One result of the stress distribution accompanying smooth muscle constriction would be a tendency for fluid movement from high-pressure regions of the submucosa to low-pressure regions. If a blood vessel were located in one of the low-pressure regions (shown in blue in Fig. 5), it would tend to be open, and the transmural pressure would favor a transfer of filtrate out of the vessel and into the interstitium. A blood vessel in a high-pressure region would tend to collapse because of a negative transmural pressure and, therefore, be unable to exchange liquid. This pattern of open and closed vessels is consistent with the observations of Wagner and Mitzner (29).

Fig. 5. Color map showing distribution of pressure within airway wall. Magnitudes range from $-0.52E_0$ (dark blue) to $+3.4E_0$ (red); zero corresponds to the boundary between light and dark blue. Applied external pressure is $0.28E_0$ where $E_0$ is elastic modulus of outer layer.
Most of the submucosa is in compression, so it follows that some submucosal fluid could be lost during smooth muscle constriction; in this case, liquid would flow into regions of lower hydrostatic pressure such as the adventitia (outside of the smooth muscle) or the airway lumen. Either could further contribute to luminal obstruction. If fluid moving into the adventitial region increased the outer airway diameter, this would tend to decouple the airway from the surrounding parenchyma, similar to that suggested by Lai-Fook et al. (15). Accumulation of liquid in the lumen further occludes the airway and increases the resistance to airflow (28).

When theoretical models, such as the one presented here, are developed, the assumptions on which the model is based must be critically appraised. We assumed that the Poisson ratio $\nu$ of the layers was 0.5, indicating an incompressible material that cannot change volume in its deformation. Our model has allowed the initial prebuckled structure to be compressed in an axisymmetric manner. This necessarily implies a reduction in the internal perimeter, which does not agree with the experimental findings of Ames et al. (11). We have measured the reduction in the internal perimeter that our model exhibits before buckling to be $<5\%$, which would not likely be detectable experimentally. Moreover, small perturbations in the model geometry, likely more representative of the actual anatomy, result in less material compression and smaller changes in internal perimeter. Recently, Sasaki et al. (26) have presented data on airway wall area changes after bronchoconstriction. They aerosolized either saline or carbachol into the lungs of 24 rabbits and, after rapid freezing with liquid nitrogen, measured the inner airway wall areas. They found no difference in the relationship between internal perimeter (a marker of airway size) and inner wall area between the airways constricted with carbachol and the unconstricted saline airways. Although this study did not partition the airway wall into the subdivisions we have used in our model, their results are supportive of the inner wall tissue being close to an incompressible material during the short time of smooth muscle constriction. It is, however, possible that over longer periods of time, or during prolonged constriction, sufficient time would exist for fluid movement; unfortunately, without information about hydraulic flow properties of the mucosa, the time required for fluid movement is unknown.

The assumption of plane strain implies no deformation in the axial direction. The computations were repeated for these simulations by using an alternative assumption of plane stress implying zero axial stress and complete freedom of axial movement. In these simulations, we obtained results that were generally similar to the plane strain calculations. The assumption of linearity in the material response is more difficult to address. The model we have presented clearly demonstrates that the bulk of the mucosal tissue is in a compressive state during smooth muscle shortening. Unfortunately, we have been unable to locate compressive stress-strain relationship data for these compliant tissues. Tensile data for tracheal membranous tissue are available from Ogawa (21) in humans and Okazawa et al. (23) in rabbits. In both of these studies, the stress-strain relationship was found to be nonlinear over a range of strain from 0 to 500%, but the local behavior up to $\sim$10–15% strain, which would be more applicable to our investigations, appeared approximately linear.

The external loading in the model, simulating the effect of smooth muscle constriction, consisted entirely of radial and circumferential stresses, not axial. This, of course, is a simplification of the true anatomic arrangement of the smooth muscle surrounding the airways. Bates and Martin (3) have used a theoretical formulation of luminal narrowing after smooth muscle constriction based on varying angles. These studies, and similar simulations by Wiggs et al. (30), have demonstrated that smooth muscle angles of 30° or less have relatively little effect on longitudinal changes in airway length. Miller (19) has measured smooth muscle angles of $\sim$15° or less, and Eina et al. (7) have shown values of $\sim$30° to the axis. These theoretical and anatomical data further support the model formulation as a plane strain problem. In addition to differences in smooth muscle angle, it is likely that the smooth muscle is not uniformly distributed about the airway wall circumference. It is interesting to note that a nonuniform stress acting on the exterior of thick-walled tubes has little effect on the final buckled structure. This implies that circumferential variation in the smooth muscle force or the presence of outward parenchymal tethers would not necessarily alter the expected buckling pattern.

The model used in this work consisted of only two layers and represents an obvious simplification of the anatomy of an airway. This formulation was selected, since it represents one of the simplest engineering structures that is expected to collapse with multiple folds, as is seen in constricted airways. The relative mechanical moduli of the layers are also of great interest, since the ratio $E_i/E_o$ certainly influences the final buckled configuration. If the inner layer is much stiffer than the outer layer ($E_i \gg E_o$) then an applied stress to the outermost edge will eventually propagate through the outer layer until the thin inner layer is compressed. At this point, the marked difference in moduli between the inner and outer layers will result in a buckling mode identical to that of an externally loaded thin ring or shell. If, in contrast, the outer layer is much stiffer then the inner layer, $E_i \ll E_o$, then the structure will collapse into the expected mode of a thick-walled tube. In both of these conditions, $E_i \gg E_o$ and $E_i \ll E_o$, the preferred buckling mode is a two-lobe (27) collapse that resembles a simple peanutlike shape. This is the configuration that would initially occur in the model of Lambert (17), in which the submucosa is treated as a fluid and, therefore, $E_i \gg E_o$. Because the two-lobe configuration is not typically seen in the constricted airways of either normal or asthmatic patients, it is reasonable to speculate that a careful
balance is maintained between the relative mechanical properties of these layers.

The two-layer composite model from which most of our results are obtained omits other components of the airway wall that could potentially influence the mucosal folding pattern. Parenchymal attachments, concentrated at specific sites around the airway circumference, might be expected to influence the folding pattern. However, when point forces of a magnitude equal to those associated with discrete parenchymal attachments are incorporated into the model, the buckling pattern is not significantly altered (data not shown). Nor are these predictions particularly sensitive to other local structures such as glands that might be found in a typical airway. The model shows that the thick submucosal layer provides damping of such disturbances, distributing their effects more uniformly over the lamina propria. In general, the buckling pattern is governed by gross dimensions and stiffnesses rather than by localized imperfections, at least provided the variations associated with geometrical or structural imperfections are not too great.

Alternative explanations for the observed buckling following smooth muscle shortening have been considered by others. Wagner and Mitzner (29) have studied the bronchial vasculature in sheep airways, noted the prominence of large vessels within the mucosal folds, and speculated that these large vessels were structurally weak and predetermine the fold location and number. Kuwano et al. (14) found a marked increase in the relative volume fraction of blood vessels in the inner airway wall of asthmatic relative to control subjects, but in the asthmatic subjects only 3-4% of the wall area was vessels. Typically, the very large blood vessels seen in sheep airways are not seen in human airways. We made some specific changes to the material properties of single elements or pairs of elements to test whether minor local weak spots in the mucosa would alter the preferred buckling mode. We found no variation from our original model and, therefore, do not believe that small blood vessels in the airway wall control the mode of buckling.

Although clearly an abstract representation of an airway wall, this model does provide some unique insights into the effects of mucosal folding. As indicated in Fig. 5, pressure levels within the inner layer are far in excess of those in the outer layer; this is also true of other stress components, including shear stress that would tend to separate the layers. This shear stress is markedly influenced in our simulations by the number of mucosal folds. Laitinen and Laitinen (16) clearly identify, as others have, that epithelial sloughing is a distinct marker of asthma. It is interesting to speculate on a possible connection between mucosal folding patterns as well as shear stress at or near the epithelium and epithelial sloughing, although we have no direct data to support this theory.

Although highly idealized, this model provides insights into the mechanisms of airway mucosal folding accompanying smooth muscle constriction. We have shown that variations in airway wall dimensions or wall material properties both can alter the buckling pattern of the structure into a different number of mucosal folds. In particular, a thickening or stiffening of a thin inner structural layer, compatible with that seen in the subepithelial collagen layer of asthmatic airways, leads to a reduced number of mucosal folds and the possible enhancement of luminal narrowing, a finding that has not been previously considered or reported.

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