Comparison of rat and mouse pulmonary tissue mechanical properties and histology

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Received 28 December 2000; accepted in final form 17 September 2001

Small rodents have been widely used to investigate the physiology and pathogenesis of pulmonary disease because large amounts of similar animals can be found, thus allowing the easy reproducibility of a variety of models of pulmonary disease. Rats and mice are easily raised and maintained, and pure-bred strains can be found. In addition, the whole lung can easily fit in one slide, thus yielding an overall morphological study. Lung parenchymal strip has been used to study the behavior of pulmonary tissue in response to different contractile agonists and antagonists, as well as to examine the tensile and viscoelastic properties of the pulmonary parenchyma (3, 21). Additionally, some studies have indicated that the connective matrix structure may determine the mechanical behavior of lung tissue (3, 9, 17). However, interspecies differences have been reported (6, 22). These facts highlight the importance of understanding how tissue mechanical properties vary among different rodents to better describe a specific disease. Finally, little information about mice lung mechanics (6), as well as mice parenchymal strips properties, is presently available (18).

To determine the possible differences between mouse and rat lung parenchymal micromechanical behaviors and whether specific lung parenchymal characteristics could be related to any mechanical profile, we analyzed rat and mouse resistance (R), elastance (E), and hysteresivity (η) during sinusoidal oscillations of lung parenchymal strips and their corresponding histopathology. The collagen-elastin matrices of both species were also studied.

MATERIALS AND METHODS

Tissue preparation. Five male Wistar rats (250–300 g) and five male BALB/c mice (25–30 g), with age and maturation matched, were sedated (diazepam: 5 and 1 mg ip, respectively) and anesthetized with pentobarbital sodium (20 mg/kg ip). Then the animals were tracheotomized, and a snugly fitting cannula (1.4 and 0.8 mm ID, respectively) was introduced into the trachea. The lungs were removed en bloc at the functional residual capacity and rinsed in a modified Krebs-Henseleit (K-H) solution containing (in mM) 118.4 NaCl, 4.7 KCl, 2.5 CaCl₂·H₂O, 0.6 MgSO₄·H₂O, 1.2 K₂PO₄, 25.0 NaHCO₃, and 11.1 glucose, pH 7.40 and 6°C. A 3 × 10-mm strip of subpleural parenchyma was cut from the periphery of each left lung. Pleural tissue was removed, and the strips were kept in a recirculating bath of iced K-H solution that was continuously bubbled with a mixture of 95% O₂-5% CO₂.

Lung strips were weighed, and their unloaded resting lengths (L₀) were determined with a caliper. Lung strip volume (vol) was measured by simple densitometry as vol = ΔF/Δs, where ΔF is the total change in force before and after strip immersion in K-H solution and Δs is the mass density of the tissue (1.06 g/cm³).

Apparatus. Parenchymal strips were suspended vertically in a K-H organ bath maintained at 37°C and continuously bubbled with 95% O₂-5% CO₂. Metal clips made of 0.5-mm-thick music wire were glued to both ends of the tissue strip with cyanoacrylate. One clip was attached to a force transducer (PT03, Grass Instruments, Quincy, MA), whereas the other one was fastened to a vertical rod. This fiberglass stick was connected to the cone of a woofer, which was driven by the amplified sinusoidal signal of a waveform generator.
Measurements of parenchymal mechanics. To calculate tissue R, E, and η, force-length curves were analyzed (9). Instantaneous average cross-sectional area ($A_i$) was determined as $A_i = \text{vol}/L_i$ (cm$^2$), where $L_i$ is instantaneous length. Instantaneous stress ($\sigma_i$) was calculated by dividing force ($F_i$; g) by $A_i$ ($\sigma_i = F_i/A_i$).

All mechanical parameters were measured cycle by cycle. Tissue R was determined from the enclosed area of force-length loops

$$R = (4 \cdot H)[\pi \cdot \omega \cdot (\Delta e)^2] \quad (1)$$

where H is the stress-strain hysteresis area, ω is the angular frequency [$\omega = 2\pi f$ (rad/s), where f is frequency], and $\Delta e$ is the normalized strain or peak-to-peak change in length divided by $L_B$. Tissue dynamic E was determined as

$$E = (\Delta \sigma_i/\Delta e) \cos \theta \quad (2)$$

where $\Delta \sigma_i$ is the peak-to-peak change in force, and θ is the phase lag between force and displacement [$\theta = \sin^{-1}[4 \cdot H/((\pi \cdot \Delta \sigma_i \cdot \Delta e))$]. The $\eta$, which is an empirically determined variable that quantifies the dependence of dissipative processes on elastic processes (3), was calculated as

$$\eta = \tan \theta \quad (3)$$

Morphometric analysis. At the end of the experiments, the organ bath was removed, and the parenchymal strips were frozen at the tension maintained during the experiment by rapid immersion in liquid nitrogen. Frozen strips were fixed in Carnoy's solution (ethanol-chloroform-acetic acid, 70:20:10) at −70°C for 24 h. Solutions with progressively increasing concentrations of ethanol at −20°C were then substituted for Carnoy's until 100% ethanol was reached. The tissue was maintained at −20°C for 4 h, warmed to 4°C for 12 h, and then allowed to reach and remain at room temperature for 2 h (17). After fixation, the tissue was embedded in paraffin. Four-micrometer-thick slices were obtained by means of a microtome. They were stained with hematoxylin-eosin.

Morphometric analysis was performed with an integrating eyepiece with a coherent system made of a 100-point grid consisting of 50 lines of known length coupled to a light microscope (Axioplan, Zeiss, Oberkochen, Germany). Sections were examined at ×400 magnification, and the fractional areas of alveolar wall (AW), blood-vessel wall (BVW), and bronchial wall (BW) were determined by the point-counting technique (7). All points falling on these components were counted and divided by the total number of points. This analysis was performed in 10 random, nonoverlapping fields in each strip. BW and BVW were counted when a point fell on the endothelial layer, the epithelial layer, the smooth muscle, or associated connective tissue. Points falling on alveolar air spaces, blood-vessel lumen, and bronchial lumen were excluded.

The tissue slices also underwent specific staining methods to characterize the collagenous and elastic system fibers in the alveolar septa. For collagen, the tissue was stained in a solution of Sirius red dissolved in aqueous saturated picric acid and observed under polarized light microscopy, as the enhancement of collagen birefringence promoted by the picrosirius-polarization method is specific for collagenous structures (15). For elastic fibers, two different methods were used: Weigert's resorcin fuchsin (RF) method (11), which sets in evidence elainin (El) and fully developed elastic fibers (FDEF), and the RF method modified with oxidation (5), which identifies the three components of the elastic fiber system [El, oxytalan (Oxy), and FDEF]. The Oxy fiber content was calculated by subtracting the amount of fibers given by the RF method from the value provided by the RF method modified with oxidation method. In each strip, 20 different microscopic fields were randomly selected to quantify collagen and elastic fibers. Quantification (×200 magnification) was carried out with the aid of a digital analysis system, using specific software (Bioscan-Optimas 5.1, Bioscan, Edmond, WA). The images were generated by a microscope (Axioplan, Zeiss, Oberkochen, Germany) connected to a camera (Trinitron CCD, Sony, Tokyo, Japan) and fed into a computer through a frame grabber (Ocula TCX, Coreco, St. Laurent, Quebec) for off-line processing. The thresholds for fibers of the collagenous and elastic systems were established after enhancing the contrast up to a point at which the fibers were easily identified as either black (elastic) or birefringent (collagen) bands. The area occupied by fibers was determined by digitized densitometric recognition. Bronchi and blood vessels were carefully avoided during the measurements. To eliminate any bias due to septum edema or alveolar collapse, the areas occupied by the elastic and collagen fibers, measured in each alveolar septum, were divided by the length of the corresponding septum. The results were expressed as the total area of elastic and collagen fibers per unit of septum length.

The lungs of five mice and five rats were fixed in Carnoy's solution and stained with hematoxylin-eosin, as described above. Histological analysis was also performed with an integrating eyepiece with a 100-point grid consisting of 50 lines of known length (1,250 μm) coupled to a light micro-
scope (Axioplan, Zeiss). Mean alveolar diameter \((L_m)\) was determined by counting the number of intercepts between the eyepiece lines and the alveolar septum of each microscopic field. \(L_m\) was expressed as the relation between the line length (1,250 \(\mu m\)) and the total number of intercepts. This analysis was performed in 10 random, nonoverlapping fields in each animal.

Statistical analysis. The normality of the data (Kolmogorov-Smirnov test) and the homogeneity of variances (Levene median test with Lilliefors’s correction) were tested. In all cases, both conditions were satisfied, and thus one-way ANOVA for repeated measurements was used to determine the effect of frequency on E, R, and \(\eta\) in each group. If multiple comparisons were required, Student-Newman-Keuls test was applied. Comparisons between E, R, \(\eta\), and morphological data between the two species were analyzed through \(t\)-test.

Multiple linear regression, with animal species as the second factor, was performed to identify the relationships between functional and morphological data. In all instances, the significance level was set at 5%.

RESULTS

The effects of different frequencies on E, R, and \(\eta\) are shown in Fig. 1. In both species, R had a negative and E a positive dependence on frequency, whereas \(\eta\) remained unchanged.

Values of E were higher in mice than in rats (24.9%), whereas \(\eta\) was larger in rats (45.3%), as shown in Fig. 1. These differences were not frequency dependent. R was similar in both species (Fig. 1).

Table 1 shows the results of the histological analysis. Although the amount of BW was similar between the two species, mouse parenchymal strips had significantly more AW \((P = 0.0008)\) and significantly less BVW \((P = 0.004)\). Mouse lungs also had smaller \(L_m\) than rats \((P = 0.001)\). The two species showed similar collagen and total elastic system fiber contents (Table 1), but mouse lung tissue had significantly more Oxy fibers and significantly less Ela and FDEF than that of the rat (Table 1).

No correlation could be established between mechanical and histological data.

DISCUSSION

This study shows that the oscillatory mechanical profiles of rat and mouse parenchymal strips differ. Although both species had similar R values, E values were higher and \(\eta\) was smaller in mice than in rats (Fig. 1). Rat and mouse lung tissue strips showed dependence of E and R on frequency, in agreement with previously reported data (2, 4, 22).

Before the results are discussed, technical issues warrant consideration. In the present study, strips could not be obtained from lungs of similarly sized animals, but the strips obtained had precisely the same size and were obtained from the same lung region. When tissue mechanics are analyzed, the morphological parameters of the parenchymal strip seem to be the major factors. In this way, the most important sample characteristic is the lung region from which the strip is obtained. The subpleural parenchymal strips are a sound model of parenchymal lung behavior, whereas in more central strips the amount of BW may play an important role in determining the hysteretic response (21). The use of instantaneous stress allows stress normalization when strips from different origins or species are compared.

Our results demonstrated similar R values in mice and rats, whereas tissue E was higher and \(\eta\) was smaller in mice than in rats (Fig. 1). Gomes et al. (6) also described a more rigid respiratory system in mice and rats at different frequencies (0.03, 0.1, 0.3, 1, and 3 Hz). Values are means ± SE of 5 animals in each group. **Different letters mean significantly different values at the various frequencies in the same group, \(P < 0.05\). *Values significantly different between groups, \(P < 0.05\).
be stressed that our study highlights the specific role of lung tissue E, because the model used is completely independent of the chest wall properties. Additionally, our results also showed smaller $L_m$ in mice than in rats.

We found that $\eta$ varied between rats and mice. In another study comparing mechanical properties among different species, Gomes et al. (6) reported that $\eta$ should be independent of animal size, because tissue damping and E had similar power variations with body weight, thus resulting in a constant ratio. However, the comparison between their results and ours is unwarranted, because the methods as well as the assumptions are different, and their results pertain to the respiratory system, whereas we are dealing with isolated lung strips.

Interestingly, taking into consideration tissue strips from various species oscillated at the same frequency and tension as ours (1, 3, 13, 22), $\eta$ varies from 0.075 (1) to 0.121 (3), $E$ ranges between 2.0 (22) and $7.4 \times 10^4$ N/m$^2$ (1), and $R$ varies from 0.1 (13) to $3 \times 10^2$ N s m$^{-2}$ (22). These variations are remarkably smaller than those presented by mechanical parameters gathered from the whole lung: pulmonary $R$ ranges between 0.98 (10) and 23 cmH$_2$O l$^{-1}$ s$^{-1}$ (19), and dynamic $E$ varies from 9.6 (10) to 336 (19) cmH$_2$O/l.

Although a statistical correlation between mechanical and histological data could not be established, our mechanical results were accompanied by a greater volume proportion of AW and smaller $L_m$ in mice than rats (Table 1). On the other hand, the anatomic makeup has a close correlation with the location from which the strip is cut from the lung, i.e., obtained from lung periphery or central regions, an important issue when constriction models are studied (17).

When different species are compared, with strips cut from the lung periphery, the volume proportion of AW can also represent different alveolar sizes among animals. In our work, this hypothesis was supported by $L_m$ values. Salerno et al. (22) reported different anatomic composition, i.e., the relative amounts of BW, BVW, and AW, when comparing rat and guinea pig parenchymal strips. These authors suggested that the volume proportion of the different anatomic structures comprising the parenchyma could play a role in the transmission of stress to the airway wall via parenchymal attachments. Haber et al. (8) showed that tissue elastic properties do not normally determine lung distensibility. They observed that it appears more likely that changes in tissue distensibility act through changes in the size of air spaces and that alteration in the density of elastic fibers may influence the distensibility to the extent that there is an accompanying change in the size of air spaces, which implicates alveolar size as the major determinant of pulmonary $E$ (8). The higher $E$ found in mice than in rats could probably be related to the smaller $L_m$ value in mice. These results are in agreement with previous suggestions (6). As our model is independent of the chest wall properties, it strengthens the possible role of the pulmonary anatomical composition in determining distensibility.

Different tissue mechanical properties between rats and mice were also accompanied by diverse extracellular matrix composition. Mice showed a higher amount of Oxy and a smaller content of elastin and FDEF than did rats (Table 1). Although some authors (16) suggest that $\eta$ does not depend on the amount of tissue participating in the expansion, our previous study on the extracellular matrix in silicotic mice (2) showed that $\eta$ was dependent on ELA + FDEF. There is no report on the relative expression of each component of the elastic system between rats and mice. The elastic system is composed of Oxy, Ela, and FDEF, defined according to increasing amounts of elastin and fibril orientation (15). The elastic properties of each fiber depend on their amorphous component, i.e., elastin (15, 20). Thus the Oxy fibers, composed solely of microfibrils without elastin, do not elongate under mechanical stress. These fibers would prevent tissue overstretching. The Ela fibers, which contain both microfibril bundles and a small content of amorphous material, are expected to display elastic properties that are intermediate between those of elastic and Oxy fibers (15). Some authors also demonstrated an important relationship between elastin and collagen in the stress-strain behavior of arteries, suggesting that an interweaving of collagen and elastic fibers permits the elastic fibers to sustain a higher stress (20). Thus the higher content of Oxy fibers and the smaller amount of the other elastic fibers could probably lead to the stiffer lung found in mice. The differences in the mechanical properties probably cannot be attributed to collagen content, because it was identical in both species.

In this way, the distinct behavior of rat and mouse $\eta$ found in the present work could be dependent on the differences in extracellular matrix composition between both species. Little is known about the mechanisms that might govern the rheology of intraparenchymal connective tissues and fiber networks. Dissipation in connective tissues may originate at the microstruc-

Table 1. Volume proportions of alveolar, blood vessel, and bronchial walls; mean alveolar diameter; collagen fiber content; total elastic system; elaunin and fully developed elastic fiber; and oxytalan fiber contents

<table>
<thead>
<tr>
<th></th>
<th>Rats</th>
<th>Mice</th>
</tr>
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<tbody>
<tr>
<td>AW, %</td>
<td>90.1 ± 0.5</td>
<td>95.2 ± 0.8*</td>
</tr>
<tr>
<td>BVW, %</td>
<td>6.2 ± 0.7</td>
<td>2.3 ± 0.6*</td>
</tr>
<tr>
<td>BW, %</td>
<td>3.7 ± 0.3</td>
<td>2.5 ± 0.5</td>
</tr>
<tr>
<td>$L_m$, μm</td>
<td>55.8 ± 1.9</td>
<td>42.6 ± 1.4*</td>
</tr>
<tr>
<td>Collagen</td>
<td>0.06 ± 0.06</td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td>Total elastic system</td>
<td>0.36 ± 0.03</td>
<td>0.42 ± 0.04</td>
</tr>
<tr>
<td>Ela + FDEF</td>
<td>0.33 ± 0.03</td>
<td>0.21 ± 0.02*</td>
</tr>
<tr>
<td>Oxytalan</td>
<td>0.05 ± 0.02</td>
<td>0.20 ± 0.06*</td>
</tr>
</tbody>
</table>

Values are means ± SE of 5 strips in each group (10 microscopic fields were analyzed in each strip). AW, alveolar wall; BVW, blood-vessel wall; BW, bronchial wall; $L_m$, mean alveolar diameter; Ela + FDEF, elaunin and fully developed elastic fiber. Collagen, total elastic system, Ela + FDEF, and oxytalan fiber contents are expressed as the total content of each fiber type per unit of septal length (μm/μm$^2$). *Values statistically different from those obtained in rats, $P < 0.05$.  

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tural level, but elasticity and \( \eta \) of the connective tissue network would appear to be more a property of the fiber matrix than of the material from which the fiber is constituted (4). The energy dissipation in such systems may be governed by contact phenomena between stress-bearing connective tissue fibers, which could occur at the molecular level within elastin or collagen fibers, 2) by shearing of the glycosaminoglycan ground substance between fibers, or 3) at surfaces of direct fiber-fiber sliding contact (4, 13, 14, 25).

In conclusion, we showed that mouse and rat parenchymal strips have diverse mechanical behaviors. The differences in tissue mechanics between both species were accompanied by different anatomical makeup and extracellular matrix composition. The results evidence a diverse proportion of specific components of the elastic system between mice and rats, which should be considered when particular diseased states are modeled.

The authors are grateful to Antônio Carlos de Souza Quaresma for skillful technical assistance.

This study was supported by Programa de Núcleos de Excelência-Ministério de Ciência e Tecnologia, Conselho Nacional de Desenvolvimento Científico e Tecnológico, Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro, Financiadora de Estudos e Projetos, Fundação Universitária Jose Bonifácio, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, and Fundação de Amparo à Pesquisa do Estado de São Paulo.

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