Rat and hamster species differences in susceptibility to elastase-induced pulmonary emphysema relate to differences in elastase inhibitory capacity

Gisella Borzone,1,4 Leonel Liberoña,1,4 Pablo Olmos,3 Claudia Sáez,2,4 Manuel Meneses,5 Tatiana Reyes,1,4 Rodrigo Moreno,1 and Carmen Lisboa1

Departments of 1Respiratory Diseases, 2Hematology and 3Diabetes and Metabolism and 4Medical Research Center, School of Medicine, Pontificia Universidad Católica de Chile; and 5Program of Pathology, Institute of Biomedical Sciences, Universidad de Chile, Santiago, Chile

Submitted 15 May 2007; accepted in final form 13 July 2007

Borzone G, Liberoña L, Olmos P, Sáez C, Meneses M, Reyes T, Moreno R, Lisboa C. Rat and hamster species differences in susceptibility to elastase-induced pulmonary emphysema relate to differences in elastase inhibitory capacity. Am J Physiol Regul Integr Comp Physiol 293: R1342–R1349, 2007. First published July 18, 2007; doi:10.1152/ajpregu.00343.2007.—Syrian Golden hamsters develop severe emphysema after a single intratracheal dose of elastase, whereas Sprague-Dawley rats exhibit mild emphysema with the same dose per kilogram body weight. We hypothesized that the development of severe emphysema is prevented in rats by the high serum level of α1-antitrypsin reported in rats, compared with hamsters, which provides for a high lung elastase inhibitory capacity (EIC). To explore this possibility, we challenged the antiprotease system of the rats by treating them with three similar weekly doses of elastase. Four months after treatment, we evaluated changes in histology, volume, and elastic properties of rat lungs and compared them with those of hamsters receiving a single dose of elastase. We also measured serum α1-antitrypsin levels and serum and lung EIC in control rats and hamsters. Results showed that, in association with 40% less serum and lung EIC compared with rats (P < 0.001), hamster lungs had upper-lobe bullae formation, severe microscopic emphysema, a fourfold increase in lung volume (P < 0.01) and a threefold increase in constant k, an index of compliance, of the lung deflation pressure-volume curve (P < 0.01). In contrast, rats developed mild emphysema, with only 50% increase in volume (P < 0.05) and 60% increase in constant k (P < 0.01). In conclusion, two species that differ in serum and lung EIC exhibit significant differences in emphysema development after elastase. Rats with high EIC, despite receiving three doses of elastase, showed significantly less derangement of morphological and physiological parameters than hamsters with low EIC receiving a single dose.

α1-antitrypsin; disease models; lung mechanics; susceptibility

The discovery of an association between emphysema and severe α1-antitrypsin (α1-AT) deficiency (23, 24) and the finding that instilled papain into the lungs of experimental animals resulted in emphysema (15), support the hypothesis that an imbalance between proteases and antiproteases plays a major role in the pathogenesis of chronic obstructive pulmonary disease (COPD) (15, 23, 24, 47). Animal models of intratracheal (IT) instillation of elastase have since been used to induce in a short period of time protease/antiprotease imbalance for the purpose of studying mechanisms involved in the pathogenesis of emphysema downstream protease release. Several investigators have shown that a single dose of elastase induces diffuse alveolar damage and rapid destruction of the alveolar septa, resulting in airspace enlargement (25, 40–43). To date, studies specifically designed to compare the magnitude and/or the pattern of elastase-induced emphysema between rodent species are unavailable, and results obtained in a particular species are often generalized to others. Species differences in susceptibility to elastase were suggested by a study done in our laboratory, showing that Sprague-Dawley rats develop only mild emphysema (3) with the dose of elastase known to produce severe panlobular emphysema in hamsters (20, 42, 43). Prompted by these findings, we performed a literature search on the increment in lung volume reported in rats and hamsters after treatment with IT elastase and found that, for the same dose of elastase per kilogram body weight, lung volume enhancement is of the order of 200–300% in hamsters (12, 20, 26, 27, 32, 35, 45) and only of 35% in rats (10, 17, 18, 20).

Species differences in susceptibility to elastase are likely to be multifactorial and are related to many independent factors specific to each one of them. However, given the specificity of α1-AT for the instilled elastase (24), we hypothesized that the development of severe emphysema is prevented in rats by the high serum level of α1-AT reported in this species compared with hamsters (19).

In a context unrelated to emphysema development, Ihrig et al. (19) showed wide differences in serum α1-AT levels among several animals, with hamsters having only 60% of the rat serum α1-AT level. These differences could result in species variations in lung elastase inhibitory capacity (EIC) and in the amount of free elastase reaching the alveolar walls. Support for this notion is provided by recent studies in tobacco smoke-exposed mice showing that the severity of emphysema development is influenced by the level of antiprotease defenses (16, 46).

Whether a larger elastase insult is capable of overriding antiprotease defenses in rats, herein inducing a more severe emphysema, is a subject that has not been addressed. The aim of our study was to challenge the antiprotease system of the rat lung, increasing the amount of instilled elastase and to evaluate whether this animal species can develop the severe emphysema-matous changes described in hamsters after a single dose (43). For this purpose, we undertook the approach of administering three subsequent doses of IT elastase to rats. We chose this repetitive approach because in a pilot study, the use of a larger
single IT dose of elastase only resulted in increased early mortality due to lung hemorrhage, with no possibility of studying later emphysematous changes, a finding reported by other investigators as well (6).

Four months after treatment with three IT doses of elastase, when emphysema was fully developed (3, 43), we evaluated changes in histology, volume, and elastic properties of the rat lungs. The magnitude of the changes observed was compared with the magnitude of those obtained in hamsters after a single dose of elastase. In addition, we measured serum α1-AT levels and its functional activity expressed as EIC in serum and in the lung of both species.

We believe that the identification of resistant and susceptible species for the development of emphysema is of great importance. Studies aimed to identify and understand protective mechanisms in rats not present in hamsters, might improve our understanding of the differences in susceptibility to cigarette smoke and other inhaled substances that damage the lungs by mechanisms involving protease release in humans.

METHODS

Animals and Treatments

The study was performed in adult male Sprague-Dawley rats (250–280 g, 50–60 days old) and in adult male Syrian Golden hamsters (90–100 g and 63–65 days old), in accordance with guidelines for the Animal Care and Use Committee at our institution. The protocol was approved by the School of Medicine Ethics Committee.

One of the problems not generally appreciated when working with porcine pancreatic elastase is the large variation in activity between different batches of the product (6). Therefore, in our study, all animals received porcine pancreatic elastase from the same batch and under equivalent environmental conditions. Fifteen hamsters received a single dose of porcine pancreatic elastase (55 U/100 g body wt in 0.3 ml saline; Sigma Chemical, St. Louis, MO), with a 40% lethality rate, occurring in the first 24–48 h. On the other hand, 30 rats received the first dose of elastase and those that survived the procedure (60%) received two extra doses 7 days apart (each dose: 55 U/100 g body wt in 0.5 ml physiological saline), with no further lethality. The dose of elastase was based on body weight, taking advantage of the power formula for predicting lung weight from body weight [lung weight = body weight^{0.99} (11)]. Because calculated lung weight of hamsters was one-third of calculated lung weight of rats, hamsters received one-third of the rat single dose of elastase. Seven hamsters and nine rats that did not receive elastase served as controls. Elastase was prepared in sterile 0.15 M saline solution immediately before administration.

Under chloral hydrate intraperitoneal anesthesia (45 mg/100 g body wt), elastase solution was directly instilled into the trachea with a 25-gauge needle after a small cervical skin incision and separation of the strap muscles. Animals were maintained in the animal care facility after recovery from anesthesia on a 12:12-h dark-light cycle, and food and water were available ad libitum.

Physiological Studies

Animal handling for physiological studies. Four months after treatment with elastase, animals were anesthetized with chloral hydrate to study the mechanical properties of the respiratory system in vivo. Mechanical properties were assessed in nonparalyzed animals that were resting in the supine position inside a small animal whole body plethysmograph. Pressure changes in the plethysmograph were detected with a Statham PM 5 pressure transducer (Statham Instruments, Hato Rey, Puerto Rico). Animals were tracheostomized via a cervical incision, a polyethylene cannula (4 and 3 mm internal diameter for rats and hamsters, respectively) was placed into the trachea and, a side port connected to a Statham PM 23 DR 300 pressure transducer for the measurement of airway pressure. A water-filled polyethylene catheter with end and side holes was placed into the distal portion of the esophagus and connected to another Statham PM 23 DR 300 pressure transducer for the measurement of esophageal pressure as an estimation of pleural pressure. Frequency response was tested for the air- and the liquid-filled catheters, and length was adjusted accordingly. The fidelity of esophageal pressure as a measure of pleural pressure was determined by the occlusion test, in which during respiratory efforts of the animal against an occlusion at the airway opening, changes in tracheal pressure were equal to changes in esophageal pressure. Before each occlusion, the catheter was flushed with 0.2 ml distilled water.

Physiological variables measured in vivo. All variables were collected into a Bio-Pac system at a speed of 30 samples/s. Transpulmonary pressure was calculated on line by subtracting tracheal pressure from esophageal pressure. Changes in lung volume were calculated from changes in body box pressure. Flow was obtained by electronic first-derivative calculation from volume and time data. From these data, tidal volume (VT), inspiratory time (TI), Ttot/Ti ratio, and Vt/Ti were calculated.

After baseline evaluation and while still in the plethysmograph, lungs were inflated to 25 cmH2O of transpulmonary pressure, and the obtained change in lung volume was defined as inspiratory capacity. Once physiological measurements were completed, animals received an overdose of intraperitoneal chloral hydrate. In rapid succession, the animal abdomen was opened and the inferior vena cava was sectioned to allow exsanguination. The animal’s trachea was then occluded at end expiratory lung volume, before opening the thoracic cage to remove the trachea and the heart-lung block.

Physiological variables measured ex vivo. After removal of the heart, the volume of saline solution displaced by the lungs was measured. End expiratory lung volume (or functional residual capacity, FRC) was calculated by subtracting the lung’s weight from the volume of saline solution displaced by the lungs using Archimedes principle (tissue density was assumed equal to 1).

Elastic behavior of both lungs cannulated at the level of the trachea was studied after inflating and deflating the lungs at tidal volume 2 or 3 times from end expiratory lung volume. The lungs were then gradually inflated with air in 1-ml steps until ~20 cmH2O pressure was reached, and gradually deflated. Fifteen seconds were allowed for pressure stabilization between steps. Two to four inflation-deflation cycles were recorded for each animal, and the best of two reproducible inflation-deflation curves, chosen by examining the resulting pressure-volume (P-V) curve, was used for results (“best” was considered to have a complete smooth inflation-deflation loop). P-V data of the deflation curve were fitted to an exponential function of the form: \[ V = A - Be^{-kP}, \]

where \( V \) is the absolute lung volume in milliliters, \( P \) is pressure in cmH2O, \( e \) is the base of natural logarithm and \( A \) is (in milliliters), \( B \) is (in milliliters), and \( \kappa \) (cmH2O^{-1}) are constants (9, 36).

Constant \( \kappa \) is an index of compliance describing the nonlinear behavior of the lung (9, 36). It reflects the rate at which the P-V curve changes its slope, thus indicating the shape of the curve. The best-fit exponential curve was obtained by an iterative least-squares method using GraphPad Prism software (version 3.02, GraphPad Software, San Diego, CA).

Histologic Assessment

Lung fixation was obtained by filling the lungs through the tracheal cannula to 25 cmH2O with 10% neutral pH buffered formaldehyde solution. The trachea was then occluded, and fixation was allowed to continue for 2–4 wk before the morphological study. Three whole left lung longitudinal sections were embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin-and-eosin for analysis by light microscopy at a magnification of ×40. A score was developed to
analyze the histological changes (Table 1). This score was previously used by us to grade the histological changes induced by a single dose of elastase in rats (3). For each of the species, the pathologist was blinded with regard to treatment. No correction was made for tissue shrinkage caused by processing.

Elastase Inhibitory Capacity and α1-AT Levels in Control Animals

Samples of lung tissue and blood from the inferior vena cava were obtained from a new group of anesthetized control rats (n = 12) and hamsters (n = 12) to measure serum α1-AT levels and serum and lung EIC.

EIC. EIC was measured both in serum and lung tissue samples by the percentage of inhibition that these samples exerted on the in vitro elastase-induced Suc-Ala-Ala-Ala-p-nitroanilide hydrolysis test, according to Bieth et al. (1).

Briefly, 40 μl of a serum dilution (1:120 for rats and 1:75 for hamsters) and 40 μl of the supernatant of a 10% fresh whole lung homogenate (pH 7.5 phosphate buffer) were used in the assay. The rate of p-nitroanilide released at 25°C was followed for 3.5 min at 405 nm (48) using a microplate spectrophotometer reader (Bio-Tek Instruments, Winooski, VT). The assays were carried out in quadruplicate. Results were expressed as units of inhibited elastase (UIE) per milliliter of serum and per gram of wet lung, respectively, calculated as described by Klumpp and Bieth (21).

Serum α1-AT levels by Western blot analysis. Total protein content of serum was measured by the method of Bradford (5). Sixty micrograms of protein were separated on a 10% SDS-PAGE and immobilized onto polyvinylidine difluoride membranes. After blocking, membranes were incubated first, with an antihuman α1-AT rabbit antibody (1:2,000, overnight at 4°C; Sigma) and then, with an anti-rabbit IgG peroxidase-conjugated goat antibody (Pierce, Rockford, IL; 1:10,000, 2 h at room temperature). α1-AT was visualized with enhanced chemiluminescence (Amersham, Piscataway, NJ) and Kodak X-ray film. Densitometric analysis was performed using the Image J 5 Program. Equal loading was controlled by Ponceau staining.

Statistical Analysis

Nonpaired nonparametric tests were used for comparisons, given the small sample size (44). Results were expressed as means ± SD. A P value <0.05 was considered statistically significant. Differences between control and experimental deflation P-V curves were analyzed in two different ways: by calculating and then comparing the constant k of the exponential curve, and by applying mixed-effects lineal models using the S-PLUS Software (22, 34).

RESULTS

Elastase Inhibitory Capacity and α1-AT Levels in Control Animals

Serum and lung elastase inhibitory capacity. Baseline EIC values obtained in the serum and lung of both species are shown in Table 2. EIC in the hamster was 2.42 ± 0.71 UIE/ml in serum and 0.9 ± 0.17 UIE/g in lung tissue. On the other hand in rats, serum and lung tissue EIC values were 4.49 ± 0.23 UIE/ml and 1.4 ± 0.20 UIE/g, respectively. Thus, serum and lung EIC values in hamsters were 54% (P < 0.0003) and 64% (P < 0.0001) of those found in rats, respectively.

Serum levels of α1-AT. The analysis of serum α1-AT protein content is shown in Fig. 1. Fig. 1A corresponds to a representative α1-AT Western blot showing that under the same conditions, the hamster serum has lower α1-AT protein content than the rat serum. The lower panel summarizes the densitometric analysis of the immune-reactive bands (n = 5), expressed as percentage of arbitrary units per milliliter of serum in the rat. It is shown that the difference in serum α1-AT protein content between rats and hamsters is of ~40%, similar to the difference obtained in serum EIC.

Histologic assessment. Figure 2 illustrates representative histological changes in hamster lung 120 days after a single dose of elastase (Fig. 2B) compared with control hamster lung showing normal parenchymal structure (Fig. 2A). Figure 2C illustrates changes induced by elastase in the four components of the histological score described in Table 1 (rules of analysis: 0, absent; 1–2, slight; 3–4, mild; 5–6, moderate; 7–8, marked; and 9–10, very marked). Elastase-treated hamsters showed marked to severe airspace enlargement and mild to moderate fragmentation of alveolar spaces and inflammation, compared

![Table 2. Species differences in elastase inhibitory capacity of serum and lung tissue](http://ajpregu.physiology.org/)

<table>
<thead>
<tr>
<th></th>
<th>Hamster</th>
<th>Rat</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum EIC, UIE × ml⁻¹</td>
<td>2.42 ± 0.71</td>
<td>4.49 ± 0.23</td>
<td>&lt;0.0003</td>
</tr>
<tr>
<td>Lung EIC, UIE × g wet tissue⁻¹</td>
<td>0.9 ± 0.17</td>
<td>1.4 ± 0.2</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SD. EIC, elastase inhibitory capacity; UIE, units of inhibited elastase.
with control hamsters that showed only slight to mild values of those parameters. The differences between control and elastase-treated groups were statistically significant. Interstitial fibrosis was not found.

Figure 3 shows representative histological findings in rat lung 120 days after three doses of elastase (Fig. 3B) compared with control rat lung showing normal parenchymal structure (Fig. 3A). Areas of mild emphysema surrounded by lung with normal alveoli are seen. Fig. 3C illustrates changes induced by elastase in the four components of the histological score described in Table 1. Emphysematous rat lung showed slight to mild airspace enlargement and fragmentation of alveolar spaces and inflammation, whereas in control rats, the score for the above-mentioned parameters ranged from absent to slight. Differences between control and elastase-treated rats were statistically significant.

Histological changes in rats receiving three doses of elastase were not significantly different from those in rats receiving a single dose of elastase (data not shown).

Physiological Studies

Changes in lung volume. Functional residual capacity (FRC) for control and elastase-treated animals is shown in Fig. 4. Consistent with the differences in severity of the morphological changes, FRC in emphysematous hamsters was four times larger than in control hamsters (3.5 ± 0.43 vs. 0.88 ± 0.41 ml, P < 0.01). In contrast, differences in FRC among control and elastase-treated rats were smaller (5.1 ± 2.3 vs. 7.7 ± 3.3 ml, respectively, P < 0.05).

Changes in lung elastic properties. Differences in in vitro lung elastic properties among control and elastase-treated animals were found in both species. However, changes in hamsters were more severe than in rats.

Fig. 3. Histological score in rats. Light microscopy (hematoxylin-and-eosin stain, original magnification ×40) of lung tissue from a control rat showing normal parenchymal structure (A) and an elastase-treated rat showing localized emphysema surrounded by lung with normal alveoli (B). C: components of the histological score in control and elastase-treated rats. Only slight to mild airspace enlargement, septa fragmentation, and inflammation are seen in emphysematous rats, whereas in controls, the score for the same parameters ranged from absent to slight. Values are presented as means ± SD. ***Significantly higher score (P < 0.001) than in controls.

AJP-Regul Integr Comp Physiol • VOL 293 • SEPTEMBER 2007 • www.ajpregu.org
Figure 5 shows the static air pressure-volume relationship during deflation of the isolated lungs of control (○) and elastase-treated animals (●). Several of the emphysematous hamster lungs had gross air leaks during inflation, coming from large bullae, which made the construction of P-V curves not possible. As seen in Fig. 5A, only four of the emphysematous hamster lungs were considered suitable for a P-V curve analysis, and all of them were inflated to less than 20 cmH₂O to prevent air leaks. The lungs of these elastase-treated hamsters showed a reduced elastic recoil pressure at isovolume than control lungs (P < 0.001, Fig. 5A).

On the other hand, all of the lungs of elastase-treated rats were suitable for inflation to 20 cmH₂O (Fig. 5B). Although the lungs of elastase-treated rats also showed an upward shift of the P-V curves (P = 0.0087), the magnitude of the shift was smaller than in the hamsters, with some degree of overlapping between control and elastase-treated rats.

Constant k of the deflation P-V curve, obtained by exponential fitting was on average, three times higher in the emphysematous hamsters than in the control group (0.625 ± 0.16 and 0.192 ± 0.04 cmH₂O⁻¹, respectively, P < 0.01). In contrast, emphysematous rats showed a modest increase in constant k (0.229 ± 0.06 cmH₂O⁻¹ compared with 0.138 ± 0.03 cmH₂O⁻¹ in the control group, P < 0.01, Fig. 6). Constant k has been shown by Schröter (37) to be independent of body mass, thus allowing interspecies comparisons of P-V curves.

Correlation Between Histological Score Components and Constant k

A significant positive correlation was found between constant k and the partial score for both airspace size (r = 0.82, P < 0.001) and septa fragmentation (r = 0.66, P < 0.001) (Fig. 7).

Table 3 summarizes the results from the study of the respiratory cycle variables at rest for control and experimental animals. Neither of the respiratory cycle parameters evaluated showed significant changes in either species. Similarly, elastase instillation did not induce significant changes in inspiratory capacity with air inflation of the lungs in vivo, in either species.

DISCUSSION

This study reveals an association between the severity of emphysema development after IT elastase instillation and elastase inhibitory capacity of serum and lung tissue. Syrian Golden hamster, with low serum and lung EIC develops a severe diffuse emphysema with bilateral upper lobe bullae formation after a single dose of elastase. Conversely, repeated doses of the protease into the lungs of Sprague-Dawley rats with higher serum and lung EIC were not capable of inducing the type of emphysema observed in hamsters. Instead, they
acquired only a mild emphysema, which was not different from that reported by us previously (3), using a single dose of elastase.

In addition to the histological differences, we describe changes in mechanical properties of the lungs of rats and hamsters after elastase treatment, an aspect not previously studied in a comparative fashion, which proved to be useful as a complement to the histological observations in the evaluation of the magnitude of the lung effects of elastase. Constant \( k \) of the pressure-volume curve was particularly useful as an index of compliance in our model, involving animals of different size and lung volume. It describes the shape of the curve, independently of both the absolute volume of the lung and the precise positioning of the P-V curve on its respective axes (8, 37). Although it is possible that constant \( k \) might have been affected by weakened, but not destroyed, alveolar walls, we believe that the increased value of this constant in our models, mostly reflects loss of alveolar walls, as shown in the histological study. Even if alveolar walls were initially not destroyed, it is likely that physiological mechanical forces acting upon weakened alveolar walls during a period of four months, might have lead to further alveolar destruction after the initial elastolytic attack. This hypothesis seems plausible since it has been shown that at least in hamsters, the rate of elastase-induced emphysematic changes is significantly reduced four months after elastase instillation (43).

Changes in mechanical properties of the lungs were significantly larger in hamsters with a single dose than in rats receiving three doses of elastase and correlated significantly with the histological changes. The increase in lung volume observed in hamsters was within the range reported in the literature for a single dose model in this species (12, 20, 26, 27, 32, 35, 45), whereas in rats, the increase in lung volume after three doses of elastase was significantly smaller than in hamsters and not different from values reported in this species after a single dose (10, 17, 18, 20). Thus, species differences in the magnitude of histological and functional changes after elastase treatment suggest the existence of protective mechanisms in the rat lung that contribute to the prevention of severe emphysema development.

Lethality rate in our study was within the range of what has been published in the very few papers that report it. We were able to find only two references reporting lethality rate after treatment with a single dose of elastase (38, 39), with values around 25%. The lack of further lethality in rats after the consecutive doses of elastase might be another reflection of the existence of protective mechanisms in the lungs of this species, which might be upregulated after the first dose.

Our interpretation of the overall results of this study is that the high serum level of \( \alpha_1 \)-AT in rats results in a high lung EIC, which, in turn, contributes to the observed resistance of this species to develop the type of emphysema seen in hamsters. Support for this interpretation is provided by the work of Blackwood et al. (2), showing that \( \alpha_1 \)-AT-deficient rats obtained by treatment with the hepatotoxic drug D-galactosamine develop more severe elastase-induced emphysema than controls.

Other important evidence relating antiprotease deficiency with susceptibility to emphysema development in animals comes from studies with the mutant pallid mouse (pa/pa), which has \( \alpha_1 \)-AT deficiency due to an abnormality in the pallidin protein that leads to an inability to secrete \( \alpha_1 \)-AT normally into the circulation (46). This mouse develops spontaneous mild emphysema late in life (28) and accelerated

### Table 3. Respiratory cycle parameters and inspiratory capacity in resting animals

<table>
<thead>
<tr>
<th></th>
<th>Control Rat</th>
<th>Experimental Rat</th>
<th>Control Hamster</th>
<th>Experimental Hamster</th>
</tr>
</thead>
<tbody>
<tr>
<td>( V_T ), ml</td>
<td>2.20±0.33</td>
<td>2.09±0.44 NS</td>
<td>0.80±0.17</td>
<td>0.84±0.29 NS</td>
</tr>
<tr>
<td>Rate, s(^{-1})</td>
<td>1.60±0.19</td>
<td>1.62±0.23 NS</td>
<td>1.22±0.25</td>
<td>1.43±0.44 NS</td>
</tr>
<tr>
<td>( T_r ), s</td>
<td>0.37±0.04</td>
<td>0.38±0.04 NS</td>
<td>0.45±0.08</td>
<td>0.4±0.08 NS</td>
</tr>
<tr>
<td>( T_I/T_{Tot} )</td>
<td>0.59±0.04</td>
<td>0.61±0.05 NS</td>
<td>0.53±0.03</td>
<td>0.54±0.08 NS</td>
</tr>
<tr>
<td>( V_T/T_i ), ml × s(^{-1})</td>
<td>5.80±0.80</td>
<td>5.50±1.20 NS</td>
<td>1.81±0.34</td>
<td>2.04±0.52 NS</td>
</tr>
<tr>
<td>IC, ml</td>
<td>14.10±2.66</td>
<td>10.67±2.76 NS</td>
<td>10.8±2.18</td>
<td>9.37±1.38 NS</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SD. \( V_T \), tidal volume; \( T_r \), inspiratory time; \( T_{Tot} \), total time; IC, volume change by inflation in vivo at 25 cmH\(_2\)O of transpulmonary pressure; NS, nonsignificant.
emphysema when exposed to cigarette smoke, a well-known protease activator, compared with mice with normal α1-AT levels.

Furthermore, it has recently been suggested that the level of α1-AT influences the pattern of emphysema development when genetically manipulated mice that differ in α1-AT serum concentration are exposed to tobacco smoke (8, 46). A renewed interest for this antiprotease has emerged since these results were obtained by long-term exposure to tobacco smoke, the main risk factor for human disease. This interest extends well beyond genetically determined emphysema and into the less severe emphysema of cigarette smokers with levels of α1-AT that are within the wide range traditionally considered normal.

The level of α1-AT could modulate emphysema development by elastase, not only by determining the level of free elastase reaching the alveolar walls, but also by modulating epithelial cell apoptosis, since it has been shown that α1-AT inhibits caspase-3 activity (33).

With regard to the magnitude of the differences in serum α1-AT levels and EIC among rats and hamsters, it could be argued that it may not be sufficient to explain the large differences in emphysema development. However, this difference in α1-AT is larger than the 30% reduction in α1-AT reported in C57BL/6J+/+ mice, known to develop emphysema more rapidly after cigarette smoke exposure than Bab/c and NMR mice (7, 8, 46). Interestingly, C57BL/6J+/+ mice are also highly susceptible to damage by silica and bleomycin, conditions in which enhanced elastolytic activity has been implicated.

It is noteworthy that the elastase model, a relatively crude one, in which lung injury is caused by a single massive insult, not only provides similar information as the continuous low-grade inflammatory process of tobacco smoke regarding the importance of α1-AT influencing the severity of emphysema development but also suggests common pathological pathways in response to different agents. In this regard, it is of interest that the level of α1-AT not only influences the pattern of emphysema both in tobacco smoke-exposed mice and in elastase-treated rodent species but also modulates lung response to IT administration of bleomycin (7), an antineoplastic agent that induces pulmonary fibrosis with an emphysematous component adjacent to the areas of fibrosis (4, 7). Whereas a greater deficit in EIC corresponds to a potentiation of bleomycin-induced lung injury (7), the administration of exogenous α1-AT reduces bleomycin-induced lung injury (31).

Applicability of data obtained in animals with IT elastase to humans with COPD has been questioned, given the acute nature of the protease insult, the availability of tobacco-smoke animal models of COPD, and the fact that most smokers do not have exclusively emphysematous changes. We believe that although IT elastase instillation might not be a good model of COPD, undeniably, it is one of acute lung injury that results in different levels of lung destruction depending, in a similar way as tobacco smoke, on the level of antiproteases.

Other mechanistic possibilities for the species differences in elastase-induced emphysema could include differences in 1) distribution and/or clearance of the instilled elastase, 2) susceptibility of elastin or some other lung matrix component to degradation, and or 3) repair processes following the elastolytic attack (14, 29, 30). Although we did not study elastase clearance, in a previous work (unpublished data), the observation of hematoxylin-and-eosin stained parasagittal sections of the entire lung at low magnification showed an even distribution of the early inflammatory reaction present in both species after elastase instillation. Although species differences in the repair process are likely to exist (14, 29, 30), the fact that challenging the repair system of the rat with serial doses of elastase did not result in a more severe emphysema, suggests that protective mechanisms preventing severe lung damage might reduce the need for repair.

Our study additionally illustrates the need for caution when extrapolating data on emphysema development in one species of rodents to another. This concept may have practical implications when comparing the results of studies designed to evaluate hyperinflation-induced adaptive changes in the respiratory muscles (20), as well as the results of therapeutic interventions such as lung volume reduction surgery.

In conclusion, our results show an association between the severity of emphysema development after elastase and the level of antiprotease defenses and provide an insight into a mechanism involved in species differences in susceptibility to lung injury by proteases. We believe that the identification of resistant and susceptible species and strains for the development of emphysema shall prompt studies designed to identify and understand new mechanisms underlying differences in susceptibility to emphysema in humans (13). In addition, studies regarding α1-AT metabolism and function in animal models with known differences in susceptibility to lung injury by several agents might reveal whether or not there is a role for this antiprotease in emphysema development in smokers.

ACKNOWLEDGMENTS

The authors thank Rodrigo Urrea for technical assistance.

GRANTS

This study was supported by FONDECYT Grants 198 0917 and 1030412.

REFERENCES


