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α_1-Antitrypsin Protects Neonatal Rats from Pulmonary Vascular and Parenchymal Effects of Oxygen Toxicity

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ABSTRACT

We investigated whether α_1-antitrypsin (α_1-AT) might protect neonatal rats from the pulmonary parenchymal and vascular effects resulting from hyperoxic exposure. Neonatal rats born into and maintained in hyperoxia (60% fraction of inspired oxygen) or room air were injected with a loading dose of α_1-AT (72 mg/kg) followed by 36 mg/kg every 72 h or with vehicle during the first 14 d of life. At the end of the experimental period, we measured body weight, lung compliance, lung volume, alveoli per mm^2, and total number of alveoli and assessed right ventricular hypertrophy and vascular changes consisting of medial hypertrophy, muscular extension into peripheral, normally nonmuscular arteries, and number of peripheral arteries relative to alveoli. Our data show that α_1-AT treatment prevented the reduced lung compliance observed in the untreated hyperoxia-exposed neonatal rats, as well as the right ventricular hypertrophy and the associated vascular changes of medial hypertrophy of muscular arteries and muscularization of distal arteries. Reduced lung compliance in the hyperoxic but α_1-AT–untreated rats was associated with a reduction in lung elastin compared with room-air or α_1-AT–treated rats. In room-air rats, α_1-AT treatment increased lung compliance but also reduced the number of arteries relative to the number of alveoli, a feature that was not, however, associated with right ventricular hypertrophy. Our data suggest that supplemental α_1-AT might restore the imbalance in elastolytic activity induced by hyperoxia and thereby alleviate the toxic effects on lung parenchymal and vascular development. (***Pediatr Res* 36: 763–770, 1994**)

Abbreviations

α_1-AT, α_1-antitrypsin
BPD, bronchopulmonary dysplasia
RDS, respiratory distress syndrome
FiO_2, fraction of inspired oxygen

BPD, the condition first described by Northway *et al.* (1) in 1967, is responsible for significant morbidity and mortality in preterm infants and is currently the most common cause of chronic respiratory illness of infancy (2). The advent of surfactant therapy for RDS brought with it the hope that fewer babies would go on to develop BPD or chronic lung disease. What has happened instead is that more preterm infants are surviving the acute illness, but the effect of surfactant on the incidence of BPD has been disappointing, with an absolute reduction of only 10.9% (3). Clearly, there is more to RDS than surfactant deficiency alone. The etiology of BPD is thought to be multifactorial, the most important risk factors including degree of prematurity, severity of RDS, duration and intensity of oxygen therapy, and mechanical ventilation, as well as pulmonary air leak. Other factors thought to be contributory include fluid overload, congestive heart failure, patent ductus arteriosus, infection, low levels of endogenous antioxidant enzymes, and vitamin deficiencies (3).

It has been appreciated for more than 20 y, however, that the interaction between protease and antiprotease mechanisms in the developing lung may play a key role in the pathogenesis of RDS and BPD (4–10). Merritt *et al.* (11) have shown that neutrophil elastase is elevated in the tracheal lavage fluid of infants with RDS who go on to develop BPD. Teleologically, the pulmonary antiprotease system exists to protect alveoli from proteolysis by proteases such as elastases (12). Elevated elastase activity may be related to the neutrophil influx that occurs in infants with RDS who go on to develop BPD (13, 14).

Proteases in the pulmonary parenchyma can digest fibrillar collagen (I and III), elastin fibers, laminin, and

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fibronectin. α_1-AT is the major modulator of elastase activity (15) and is the only antiprotease that has been extensively studied in the preterm infant (12). Although levels of α_1-AT may be normal in preterm infants, exposure to high oxygen to treat respiratory failure may result in inactivation of the methionine site of α_1-AT (16). Thus, there may be a place for supplemental α_1-AT in the treatment of preterm infants at risk for BPD.

In this study, we therefore investigated the efficacy of α_1-AT given by infusion to prevent abnormal lung parenchymal and vascular development in rat pups subjected to elevated oxygen concentrations from birth. The features and the time course of the normal early growth and remodeling of the arteries and alveoli of the newborn rat lung have been described by Meyrick and Reid (17). Essentially, alveolar multiplication is largely observed between 3 and 8 d of life, whereas arterial concentration, constant from birth until d 8, doubles between 8 and 11 d. Our previous studies suggest that there is an additional increase in alveolar multiplication between 5 and 7 wk of life (18). The medial wall thickness of muscular preacinar arteries doubles during the second week of life but decreases with increasing size or external diameter of the vessel. Maturation of the newborn rat lung, which includes an increase in alveolar number, pulmonary arterial size and number, and a decrease in arterial medial wall thickness, appears to depend on elastic tissue remodelling, a process which, it has been speculated, is characterized by a coordination of elastin synthesis and elastolysis (16).

Previous studies in our laboratory (18), as well as by others (19–22), have demonstrated impaired lung growth in infant rats exposed to hyperoxia in that the numbers of alveoli and arteries are significantly decreased relative to values in normoxic rats. Exposure of adult rats to hyperoxia decreases alveolar concentration and produces vascular changes similar to those observed in rat pups, *i.e.* muscular extension into peripheral arteries and loss of small arteries (23). This hyperoxic injury has been correlated with the appearance of increased collagen rather than elastin degradation products in bronchoalveolar lavage fluid, suggesting increased activity of collagenases rather than elastases (24). However, what has been noted in the developing lung of hyperoxic newborn rats, as well as in infants who develop BPD, is an impairment of alveolar growth that has been linked to significant elastin remodeling (25, 26).

Oligohydramnios and smoke inhalation are two additional experimental conditions associated with a reduction in infant rat lung elastin content and impairment of alveolar development (27, 28). Furthermore, experimentally induced elastolytic injury of infant (29) and adult (30) rat lungs produces alveolar destruction and impairment of new growth. All these studies provide evidence that elastin synthesis and elastolysis play an important role in the development of the lung.

Therefore, we hypothesized that augmentation of the newborn rat's own elastase-inhibiting capability by administration of exogenous human α_1-AT may protect the developing lung from hyperoxic injury, as well as minimize the associated vascular changes.

METHODS

Experimental design. The study design consisted of 40 Sprague-Dawley rat pups from four different litters. Ten pups were studied in each of four groups: *1*) room air/vehicle, *2*) room air/α_1-AT, *3*) hyperoxia/vehicle, and *4*) hyperoxia/α_1-AT. Pregnant dams were exposed to hyperoxia (60% oxygen at a flow rate of 10 L/min) or to room air in 6-ft^3 Plexiglas chambers on the day before anticipated delivery. Randomization to room-air or hyperoxia exposure was performed the day before delivery of the pups. Rat pups born in hyperoxia were continually exposed for the first 14 d of life. The oxygen (Miniox I gas analyzer, Catalyst Research, Owings Mills, MD) and carbon dioxide concentrations (Medical Gas Analyzer LB-2, Beckman, Schiller Park, IL) in the chambers were monitored daily. Rat pups in the litters were randomly designated either α_1-AT or vehicle and then separated, and the dams were alternated between room air and hyperoxia. This was performed to prevent maternal oxygen toxicity and death. The rat pups were administered either an intraperitoneal dose of α_1-AT or an equal volume of saline within 1 h of birth and then at intervals of 72 h until the completion of the study on d 14. The initial loading dose was 72 mg/kg and was followed by maintenance doses of 36 mg/kg. These doses were calculated using first-order elimination pharmacokinetics on the basis of extrapolation from studies in humans and animals (31–34) and our previous studies in adult rats with hypoxia-induced pulmonary hypertension (35). Our predicted endpoint was a 2-fold increase in the plasma concentration of α_1-AT, given the adult rat concentration of 11 ± 0.9 mg/L (34). We also anticipated a similar increase in lung levels over baseline, given the relative lung tissue concentration of 10% total injected cpm radiolabeled [^{125}I]α_1-AT as documented in rabbits (33).

The α_1-AT used was Prolastin (Cutter Biologicals, Miles Canada, Inc., Etobicoke, Ontario, Canada, a gift of Mary Ann Lark and Stan Beck), a sterile lyophilized preparation of partially purified human α_1-AT obtained from pooled plasma of normal donors (31). Using United States Pharmacopeia guidelines, all lots of Prolastin were routinely tested in eight rabbits for pyrogenicity and excluded from use if any rabbit showed an increase in temperature of 0.5°C or greater.

Rat pup growth. At the end of the experimental period, the pups were weighed. Because of concerns related to growth of the room-air rat pups treated with α_1-AT, we compared growth rates in two additional groups of room-air rat pups ($n = 9$ in each); one group was treated with α_1-AT, the other with vehicle. With respect to the two additional groups, the dams were rotated between treatment and control groups, and rat pup weights were obtained on the first day of life and every 3 d thereafter until

d 14. In the previous study, the dams were rotated between oxygen and room air but not between treatment and control groups, so some of the α_1-AT–associated reduction in rat pup weight may have been related to differences in the dams.

Assay for α_1-AT levels in rat blood. At the end of the 14-d experimental period, all rats were killed with an overdose of sodium pentobarbital (300 mg/kg), and 0.5-mL blood samples were removed by direct cardiac puncture for measurement of α_1-AT after which the heart and lungs were removed. Levels of α_1-AT were obtained by Laurell rocket assay (36), as previously described, using an antibody that only reacts with human α_1-AT (37). Thus, levels could be detected only in rats that had received the injections of human α_1-AT. The lung distribution would be approximately 10% of the injected dose (33).

Measurement of lung compliance. Separate groups of rat lungs were then either used for lung compliance or lung morphometry studies. Lung compliance was measured in lungs from five rats from each group by performing a tracheostomy (with polyethylene tubing, PE-60) and degassing the lung. A three-way stopcock was connected to the tracheostomy, a syringe, and an electronic transducer. Air was introduced in increments of 0.2 mL to a total volume of 1 mL, and the corresponding inflation pressures were recorded (Gould Recorder ES 1000, Gould, Inc., Cleveland, OH). Linear regression analysis was performed for the data from each animal to calculate the resulting lung volume for a given pressure, and this value was then corrected for body weight.

Preparation of heart and lungs for morphometry. Cannulation of the right pulmonary artery with polyethylene tubing (PE-20) was performed through the right ventricle, and the pulmonary vasculature was perfused rapidly with either hot (60°C) radiopaque barium gelatin until it produced a "snowflake" appearance on the pleural surface or, alternatively, 10% buffered formalin. A tracheostomy was performed with polyethylene tubing (PE-60), and the lungs were slowly distended by hand with an intratracheal infusion of 10% buffered formalin, until the pleural surfaces "wept." The barium-perfused lungs were used to assess the vasculature, the formalin-perfused lungs to assess the parenchyma. After fixation, the heart was separated from the lungs. The right ventricular free wall was dissected free from the left ventricle with septum and each was dried and weighed separately (38). The volumes of the right lungs were measured by displacement of water (18, 39).

Lung morphometry. A 1-cm^2 block of tissue was dissected from the midportion of the fixed lung, and 5-μm sections were then cut for staining by the elastin Van Gieson method. Using a microscopic grid measuring 0.2 mm^2, the number of alveoli were counted at 250× magnification in 10 randomly selected fields. The mean number of alveoli per mm^2 was determined for three rats in each group. A shrinkage factor of 1.32 (18) was applied to the alveolar concentration, *i.e.* number of alveoli/mm^2, to calculate the absolute alveolar number based on the following formula (40, 41):

$$\text{Alveolar number} = \left(\frac{\text{corrected alveolar concentration}}{1.55 \times \sqrt{p}}\right)^{3/2} \times \text{lung volume}$$

where 1.55 is the spherical constant for an alveolus and p is the volume proportion of alveoli derived by point counting the microscopic section (42).

Morphometric assessment of alveolar wall elastin on lungs stained by the Van Gieson method was also performed on three rats from each of the four groups. At a magnification of 250×, 25 fields were selected at random and analyzed using an eyepiece micrometer with a point-counting grid (Zeiss, Jena, Germany) (42). The proportion of lung alveolar wall and alveolar duct septal elastin was expressed as a percentage of total point counts of alveolar duct and alveolar wall septa.

Vessel morphometry. Arteries were identified on the basis of their accompanying airways and classified accordingly as preacinar, terminal bronchiolus, respiratory bronchiolus, alveolar duct, and alveolar wall, as well as muscular or nonmuscular. Using an eyepiece micrometer, the external diameter of each respiratory and terminal bronchiolus artery was measured and the percent wall thickness calculated according to the following formula:

$$(2 \times \text{wall thickness/external diameter}) \times 100 = \text{percent wall thickness}$$

Medial wall thicknesses were counted for 25 vessels at the terminal bronchiolus level and for 25 vessels at the respiratory bronchiolus level in three different rat pups in each group.

The percentage of fully muscularized arteries at the alveolar duct and at the alveolar wall level was also assessed in lung sections from three different rat pups in each group. We next calculated in each lung section from three different rats in each group, in 10 different fields, the number of arteries per 100 alveoli.

Analysis of data. Data from each of the four groups were compared by analysis of variance followed by Tukey's test to determine which specific groups were different. Nominal data on distal arterial muscularization were analyzed by χ^2 test. Comparisons with p values less than 0.05 were considered significantly different.

RESULTS

Growth. There was a slight (<5%) but significant increase in final body weight comparing vehicle/hyperoxia with vehicle/air rat pups. A 10–15% reduction in body weight was, however, observed comparing the two α_1-AT groups (room air and hyperoxia) with the two vehicle groups ($p < 0.01$ for both comparisons) (Fig. 1*A*). The latter difference may have reflected interdam variability, because growth curves in two additional room-air groups of rat pups in which dams were rotated showed no significant influence of α_1-AT treatment on weight gain by d 14. There were, however, slight, albeit significant,

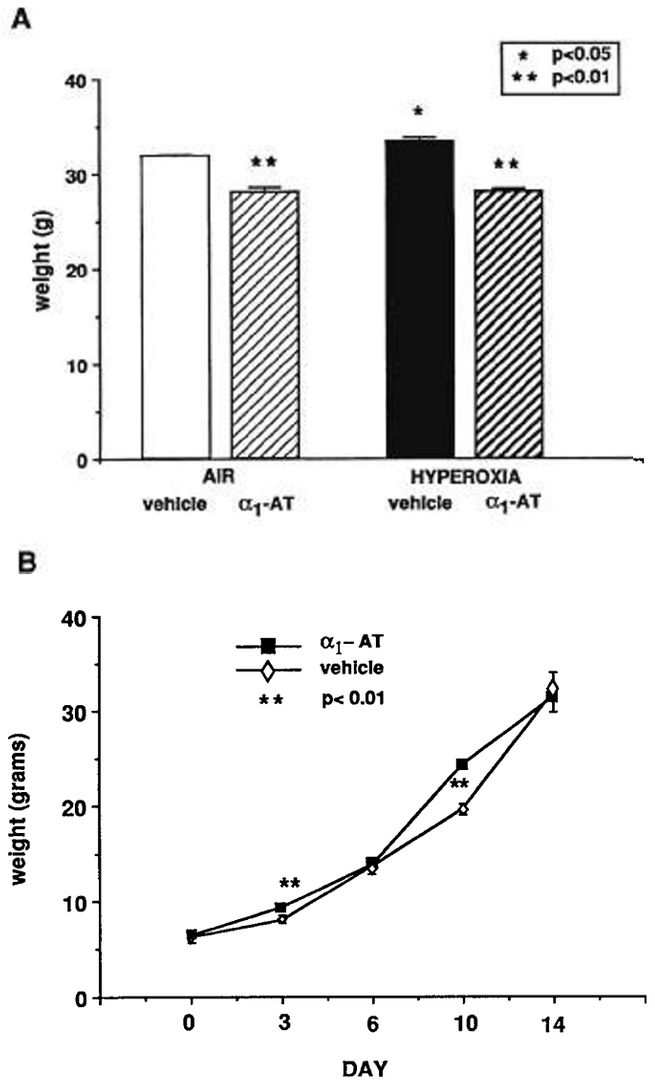

Figure 1. *A*, Graph depicting body weights at the end of the experimental period. The data show a significant increase in weight in hyperoxia/vehicle rats relative to room-air/vehicle rats ($p < 0.05$). There is a reduction of weights in the α_1-AT groups *vs* the vehicle groups ($p < 0.01$). Values are mean ± SEM; $n = 12$ rats per group. *B*, Growth curves of two room-air rat groups show no difference comparing treatment with α_1-AT or vehicle. Although there were small but significant reductions in body weight in the α_1-AT group compared with the vehicle group on d 3 and 10 ($p < 0.01$), weights were similar at d 14. Values are mean ± SEM; $n = 9$ rats per group.

decreases in weight apparent on d 3 and 10 in the α_1-AT compared with the vehicle group ($p < 0.01$ for each comparison) (Fig. 1*B*).

α_1-AT levels. We were able to detect human α_1-AT in the plasma samples from five of 10 rats receiving this agent and in none of the rats receiving vehicle. Values were at the limit of detection of our assay and ranged from 20 to 42 mg/L. Our inability to detect human α_1-AT or to detect only trace amounts after 24 h might reflect degradation after complexing with proteinases.

Lung compliance. Analysis of pressure-volume curves showed no differences among the groups at pressures lower than 4 mm Hg (0.53 kPa) and a reduction in compliance in the hyperoxia/vehicle rats *versus* the room-air/α_1-AT group at 4 mm Hg (0.53 kPa) and relative to all other groups at pressures of 5–9 mm Hg (0.67–1.20 kPa) ($p < 0.01$). It was of interest that the room-air/α_1-AT group had significantly greater compliance than both the hyperoxia/α_1-AT and the room-air/vehicle groups at pressures from 5 to 9 mm Hg (0.67–1.20 kPa). The hyperoxia/α_1-AT and the room-air/vehicle groups were similar. Thus, it seems that the reduced lung compliance observed with hyperoxia is prevented by α_1-AT, but treatment of room-air rats with this agent produces a lung with increased compliance (Fig. 2).

Lung morphometry: parenchyma. The reduced lung compliance in the hyperoxia/vehicle group was associated with a reduced content of elastin in the lung parenchyma relative to the room-air/α_1-AT group ($p < 0.05$) with the room-air/vehicle and hyperoxia/α_1-AT groups being similar and intermediate with respect to the assessment of elastin content (Fig. 3). Examination of the histologic sections revealed the previously described alterations in the distribution and structure of individual elastin fibers, including coarsening and tortuosity in the hyperoxia/vehicle lungs (25).

There were no significant differences among the four groups in morphometric assessments of lung volumes by water displacement, in alveoli per mm^2, or in total number of alveoli (Table 1). Because the evaluations were done on three animals in each group, the trend toward a reduced number of alveoli of increased size in the α_1-AT–treated room-air group may have become significant, given the power calculation of 20–30%.

Right ventricular weights. There was significant right ventricular hypertrophy as judged by the ratio of right

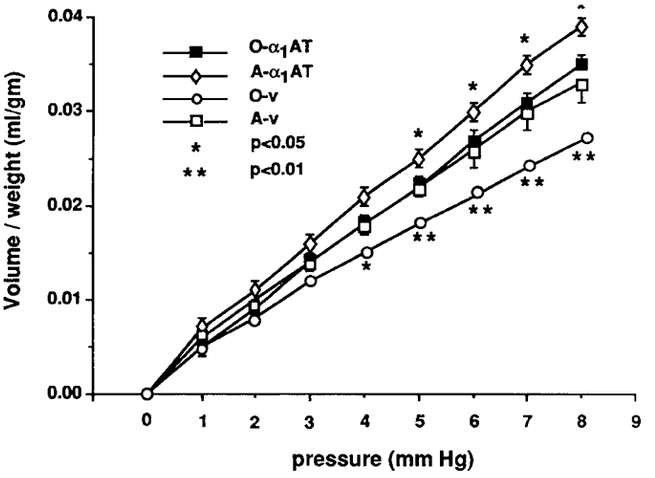

Figure 2. Graph of lung compliance assessed as pressure-volume curves corrected for body weight. *O*, hyperoxia; *A*, room air; *v*, vehicle. The data show a decrease in lung compliance in the hyperoxia/vehicle group relative to the room-air/α_1-AT group at pressures as low as 4 mm Hg (0.53 kPa) ($p < 0.05$) and a decrease compared with all other groups at pressures higher than 5 mm Hg (0.67 kPa) ($p < 0.01$) (1 mm Hg = 0.133 kPa). An increase in compliance in the room-air/α_1-AT group compared with the other groups is observed at pressures of 0.67 kPa and greater ($p < 0.05$). Values are mean ± SEM; $n = 5$ rats per group.

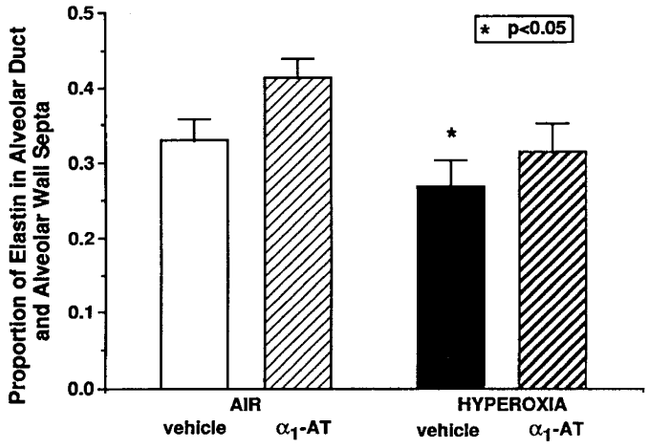

Figure 3. The proportion of elastin in alveolar duct and wall septa. There is a significant decrease in the hyperoxia/vehicle group compared with the room-air/α_1-AT group ($p < 0.05$) with the other groups being intermediate. Values are mean ± SEM; $n = 20$ fields per group.

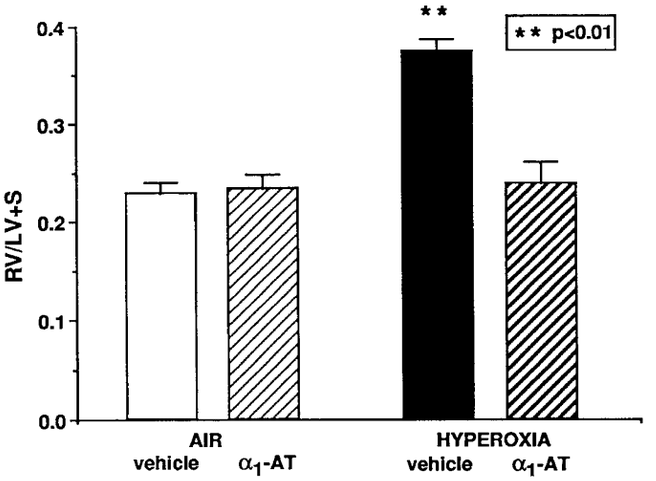

Figure 4. Graph of right ventricular weight (*RV*) compared with that of left ventricle and septum (*LV+S*). There is a significant increase in this measurement (an index of right ventricular hypertrophy) in the hyperoxia/vehicle group compared with the other three groups, room air/vehicle, room air/α_1-AT, and hyperoxia/α_1-AT ($p < 0.05$). Values are mean ± SEM; $n = 5$ rats per group.

ventricle to left ventricle and septum in the hyperoxia/vehicle group compared with the other three groups, which were similar ($p < 0.01$) (Fig. 4). Thus, α_1-AT prevented the development of right ventricular hypertrophy during exposure to hyperoxia.

Morphometry of pulmonary vasculature. Structural changes in the peripheral pulmonary arteries correlated with right ventricular hypertrophy in the hyperoxia/vehicle rat group. The percent wall thickness of muscular arteries at respiratory bronchiolus and terminal bronchiolus levels was increased, reflecting medial hypertrophy in the hyperoxia/vehicle group compared with the other groups ($p < 0.05$) (Fig. 5). That is, the pups exposed to hyperoxia and treated with α_1-AT were protected from developing this vascular abnormality. Furthermore, the percentage of muscularized alveolar duct and wall arteries was significantly higher in the hyperoxia/vehicle group (45%) compared with the hyperoxia/α_1-AT–treated (13%) or room-air/vehicle and room-air/α_1-AT pups (15 and 23%, respectively) ($p \leq 0.02$, χ^2). The number of arteries relative to the number of alveoli was significantly increased in the room-air/vehicle pups relative to the other three groups, with the greatest reduction (approximately 30%) in the hyperoxia/vehicle group ($p < 0.05$) (Fig. 6). This suggests that α_1-AT may not completely overcome the reduction in arterial concentration observed with hyperoxia; this may be related to an independent effect of this agent, because a reduction in arterial concentration is also observed in the room-air/α_1-AT group relative to the room-air/vehicle group. The magnitude of the reduction is, however, less than 20% and does not appear to be of hemodynamic significance, inasmuch as it is not associated with right ventricular hypertrophy.

Table 1. *Measurements of lung volume, number of aveoli, and size of aveoli**

	Right lung volume (mL)	Total number of alveoli × 10^5	Size of aveloi (number per mm^2)
Room air			
Vehicle	0.49 ± 0.03	60.10 ± 2.66	111.77 ± 9.89
α_1-AT	0.59 ± 0.04	45.00 ± 8.19	102.80 ± 6.76
Hyperoxia			
Vehicle	0.57 ± 0.03	52.36 ± 10.78	95.87 ± 21.86
α_1-AT	0.54 ± 0.01	52.22 ± 6.57	104.17 ± 9.30

* Values are mean± SEM; $n = 5$ for lung volume and $n = 3$ for total number of alveoli and size of aveloi as described in Methods.

DISCUSSION

We treated rats newly born into hyperoxia or room air with injections of α_1-AT. Compared with the untreated group, the room-air controls treated with α_1-AT had a reduction in arterial number relative to alveolar number that was not, however, of hemodynamic significance, at least as judged by lack of right ventricular hypertrophy. The lungs were more compliant, but morphometric assessment showed only trends toward increased elastin and a reduced number of alveoli of increased size that were not statistically significant, albeit in a small sample size. There was also a tendency toward reduced weight gain that did not seem to be a consistent feature of the α_1-AT treatment. There was no influence of α_1-AT on right ventricular weight or arterial muscularity in room-air animals. Rats treated with α_1-AT during hyperoxia, however, did not have impaired lung compliance and reduced lung elastin, as seen in untreated hyperoxia-exposed pups, nor did they have right ventricular hypertrophy and associated vascular changes of increased muscularization of peripheral arterial and increased wall thickness of muscular arteries.

Loosli and Potter (43) characterized the elastic fiber system of the lung as the key to the determination of its architecture. According to the "fishnet" theory, the elastin in the developing lung, essentially confined to the mouths of alveoli, acts as a framework on which alveoli

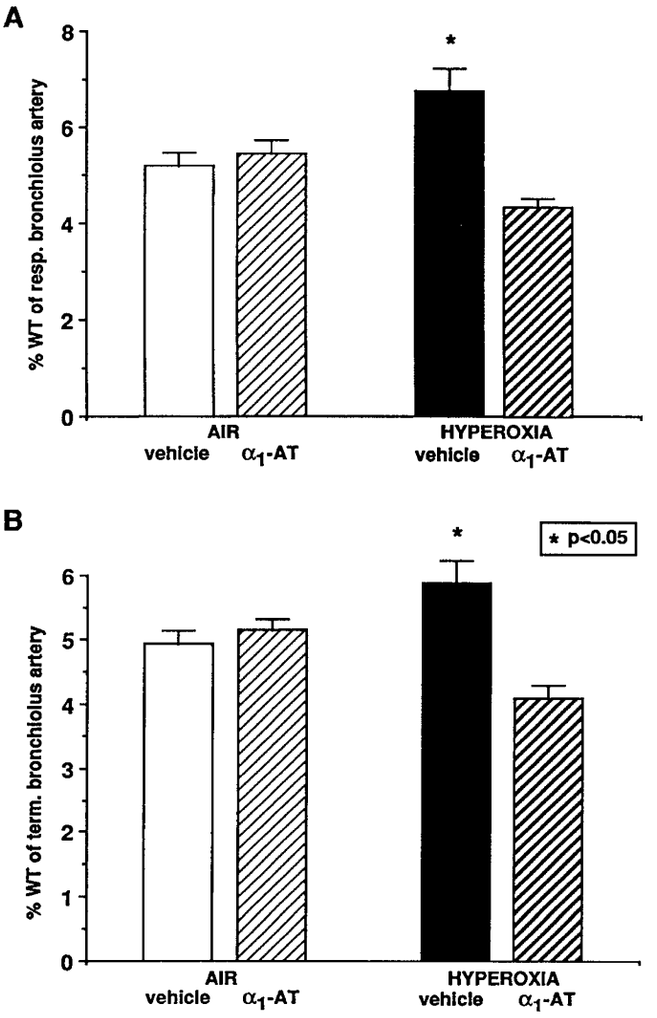

Figure 5. Graphs of medial wall thicknesses assessed as a percentage of wall thickness (*%WT*). There is a significant increase in medial hypertrophy in the hyperoxia/vehicle group compared with the other three groups, room air/vehicle, room air/α_1-AT, and hyperoxia/α_1-AT ($p < 0.05$). Values are mean ± SEM; $n = 25$ vessels per group at the respiratory (*resp.*) bronchiolus level (*A*) and 25 vessels per group at the terminal (*term.*) bronchiolus level (*B*).

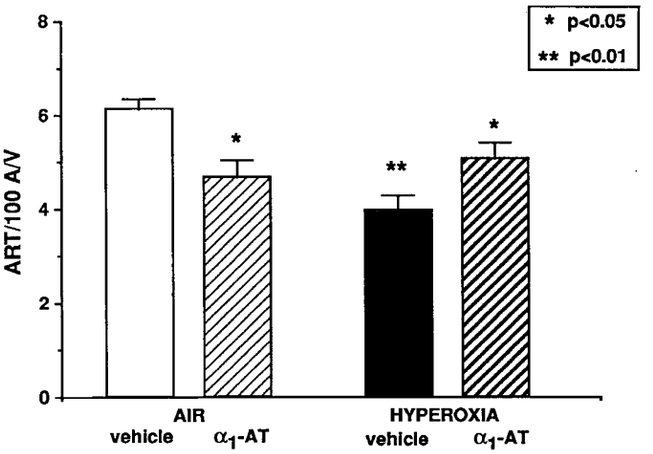

Figure 6. Graph of arteries per 100 alveoli (*ART/100 A/V*). There is a significant reduction in the hyperoxia/vehicle group compared with the room-air/vehicle rat group ($p < 0.01$). Rat groups treated with α_1-AT also show a reduction ($p < 0.05$) relative to vehicle groups. Values are mean ± SEM; $n = 30$ fields per group.

evolve from immature saccules (44). We speculated that the process of remodeling of alveoli might involve not only elastin synthesis but also limited elastolysis and that it might therefore be possible to augment alveolar number by changing the balance of elastases to elastase inhibitors in favor of the latter by their exogenous administration.

Injury to the developing lung connective tissue, especially elastin and collagen, occurs when an imbalance exists between the production of proteases and the capacity of inhibitor proteins to neutralize them (12). Preterm infants have lower levels of serum proteins, including the antiproteinases, than term infants or adults. Evans *et al.* (4) found a decrease in trypsin inhibitory protein in the cord blood of preterm infants with RDS. Infants with the most severe RDS had the lowest trypsin inhibitory capacity at d 1 of life (45). Moreover, infants with RDS are treated with $FiO_2 > 0.40$, which has been shown to cause oxidative inactivation of α_1-AT (16).

Inflammation is also known to play a role in the pathogenesis of BPD (11). An influx of neutrophils is noted between 48 and 96 h and disappears by the end of 1 wk, except in infants who go on to manifest signs of BPD (10). Kawano *et al.* (46) demonstrated a significant improvement in pulmonary function in a rabbit model of RDS rendered neutropenic by pretreatment of the rabbits with nitrogen mustard. Additional evidence for the role of inflammatory cells in the development of BPD is the fact that Merritt *et al.* (11) found a 20-fold increase in cell number in tracheal aspirates from babies who went on to develop BPD compared with those with an uneventful recovery. This increase in cellularity persisted as long as 6 wk after initiation of mechanical ventilation. Neutrophils represented more than 90% of cells at d 7.

Neutrophils secrete a serine elastase that results in the hydrolysis of extracellular matrix components, as well as immunoglobulins, fibrinogen, and complement (47). Proteolysis is necessary for the breakdown of hyaline membranes, but the uncontrolled action of elastase will have adverse effects on lung morphogenesis (12). Desmosines, elastin degradation products, have been measured in the urine of infants with RDS. Significantly greater amounts of desmosines were found in the urine of infants exposed to FiO_2 greater than 60% who developed BPD than those exposed to less than 40% who did not develop BPD (48).

We chose a hyperoxic exposure of 60% in our experimental studies on the basis of evidence that this degree of hyperoxia resulted in histologic and biochemical abnormalities in the newborn rat lung with low mortality (49). In separate studies, using the Leder stain to detect neutrophils, we found no evidence of accumulation in the pup lungs at 4, 6, and 14 d of hyperoxic exposure, although an influx of neutrophils may have occurred earlier. Thus, we did not establish in the rat pup lungs the source of the hyperoxia-induced increased proteolytic (elastolytic) activity.

The degree of hyperoxic injury to which the pups were subjected caused reduced lung compliance, although we could detect no significant reduction in lung volume, alveolar concentration, or absolute alveolar number. Although dose relationships with oxygen effects are less than clear cut, the fact that our experiments revealed no differences in these outcome parameters is at least consistent with the findings of our earlier studies, in which we exposed rat pups beginning at 10 d to a higher FiO_2 (80%). This exposure did not produce a significant reduction in lung alveolar number until the sixth week of exposure (18). A concentration of 80% oxygen from birth has not resulted in poor survival of the litters (our unpublished observation).

Based on the work of Emery and Fagan (44), the appearance of increased amounts of alveolar wall elastin in the room-air/α_1-AT lungs suggests that, with more time, these extra elastin fibers may have formed the mouths of additional alveoli. The compliance studies did reveal a significant decrease in compliance in the hyperoxia-vehicle group, as well as an increase in compliance in the group receiving α_1-AT in room air. The compliance in the hyperoxia/α_1-AT group was similar to that in the room-air/vehicle group. Lung compliance did not correlate with differences in fixed lung volume, perhaps owing to the relative insensitivity of the latter measurement.

Compliance may be impaired by abnormalities in the configuration and distribution of elastin fibers (25). Treatment with α_1-AT may, by augmenting endogenous elastase-inhibiting capacity, protect the newborn rat lung from hyperoxia-induced increases in elastase activity and assure normal lung parenchymal development. When α_1-AT is, however, given to pups breathing room air, this may inhibit the normal basal levels of elastase activity, resulting in a distribution of elastin fibers that renders the lung more compliant. It is also possible that the relative increase in compliance in the α_1-AT–treated room-air and hyperoxia groups may be related to α_1-AT preventing the inactivation of surfactant.

The ability of elastin fibers in a given tissue to stretch is limited by the amount of collagen in that tissue (50). Kida *et al.* (29) demonstrated an inverse relationship between collagen and elastin in the lungs of neonatal rat pups treated with intraperitoneal elastase. Their studies revealed increased collagen relative to elastin degradation. Compliance in their model was enhanced with elastin degradation, but this may represent a concomitant increase in the amounts and types of collagen degradation in their model of elastase-induced lung injury compared with that induced by hyperoxia.

Hyperoxia-vehicle pups compared with room-air controls had a reduction in the number of arteries relative to the number of alveoli, as we have previously observed (18). A reduction in the number of arteries relative to the number of alveoli, although less severe, was also observed in both α_1-AT–treated hyperoxia and room-air pups compared with room-air controls. This may reflect differences between litters in the timing of rapid arterial multiplication, but if treatment with α_1-AT truly reduced arterial concentration, the reduction was not severe enough to result in increased pulmonary vascular resistance, at least as assessed indirectly by measurements of right ventricular hypertrophy. Moreover, if α_1-AT does cause a reduction in arterial concentration, then this does not seem to be compounded by the effects of hyperoxia.

Vehicle-treated pups exposed to hyperoxia underwent vascular changes, including a significant increase in arterial wall thickness at the respiratory and terminal bronchiolar levels. This finding was not observed in pups treated with α_1-AT. Moreover, peripheral extension of muscle was noted to be greatest in the hyperoxia/vehicle group. The result of these vascular changes was the development of right ventricular hypertrophy in the hyperoxia/vehicle group, likely a reflection of the pulmonary hypertension that also occurs as a consequence of BPD in human infants (51).

Classic α_1-AT deficiency is not increased in incidence in infants developing BPD. However, reduced synthesis of α_1-AT compatible with immaturity or increased inactivation of α_1-AT by oxidation is known to occur (16). Dexamethasone therapy for BPD has been shown to decrease by 45% the elastase activity detected in bronchoalveolar lavage without altering α_1-AT (15). Yoder *et al.* (52) demonstrated that dexamethasone treatment resulted in a significant decrease in the requirement for respiratory support, improved pulmonary function, and reduced pulmonary inflammation indices expressed as neutrophil counts, elastase/α_1-AT, albumin, and fibronectin. This provides additional evidence implicating an imbalance between elastase and elastase inhibitors in the pathogenesis of BPD while documenting efficacy in ameliorating the course of BPD by restoring the balance through dexamethasone therapy. Alternatively, a more favorable balance may be restored by augmentation of elastase inhibitors.

α_1-AT, a blood-derived product, has been in use in adults with α_1-AT deficiency since 1987 with encouraging preliminary results (31, 53, 54). The same investigators have used recombinant α_1-AT administered as an aerosol (55). Cystic fibrosis, a disease in which patients have normal circulating levels of α_1-AT but excessive lung elastase activity, is another chronic obstructive pulmonary disorder in which aerosolized α_1-AT therapy has been used (56). Augmentation therapy has thus far been shown to be safe, with only minor reactions reported. However, the long-term effects of this therapy on the course of chronic lung disease still remain to be determined.

In a review of protease-antiprotease interactions in the immature lung, Merritt and Hallman (12) stated that the therapies designed to prevent or ameliorate BPD may require that they be instituted before or soon after the induction of elastase or the inactivation of endogenous antielastase to prevent structural alteration of lung connective tissue and basement membranes. This is what we have attempted to accomplish in our experimental model.

Our findings indicate that early α_1-AT augmentation therapy in rats exposed to hyperoxia protects them from developing anticipated pulmonary and vascular changes and right ventricular hypertrophy.

These results provide further insight into the multifactorial pathogenesis of BPD and suggest that the clinical use of α_1-AT augmentation therapy could ameliorate the course of preterm infants at risk for developing BPD.

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