Changes in proteoglycans and lung tissue mechanics during excessive mechanical ventilation in rats

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Al-Jamal, Rehab, and Mara S. Ludwig. Changes in proteoglycans and lung tissue mechanics during excessive mechanical ventilation in rats. Am J Physiol Lung Cell Mol Physiol 281: L1078–L1087, 2001.—Excessive mechanical ventilation results in changes in lung tissue mechanics. We hypothesized that changes in tissue properties might be related to changes in the extracellular matrix component proteoglycans (PGs). The effect of different ventilation regimens on lung tissue mechanics and PGs was examined in an in vivo rat model. Animals were anesthetized, tracheostomized, and ventilated at a tidal volume of 8 (VT8), 20, or 30 (VT30) ml/kg, positive end-expiratory pressure of 0 (PEEP0) or 1.5 (PEEP1.5) cmH2O, and frequency of 1.5 Hz for 2 h. The constant-phase model was used to derive airway resistance, tissue elastance, and tissue damping. After physiological measurements, one lung was frozen for immunohistochemistry and the other was reserved for PG extraction and Western blotting. After 2 h of mechanical ventilation, tissue elastance and damping were significantly increased in rats ventilated at VT30PEEP0 compared with control rats (ventilated at VT8PEEP1.5). Versican, basement membrane heparan sulfate PG, and biglycan were all increased in rat lungs ventilated at VT30PEEP0 compared with control rats. At VT30PEEP0, heparan sulfate PG and versican staining became prominent in the alveolar wall and airspace; biglycan was mostly localized in the airway wall. These data demonstrate that alterations in lung tissue mechanics with excessive mechanical ventilation are accompanied by changes in all classes of extracellular matrix PG.

The ECM is composed of collagen and elastin fibers as well as an amorphous “ground substance.” This ground substance is largely composed of proteoglycans (PGs) and glycoproteins. PGs are a heterogeneous family of molecules that consist of a core protein to which one or more glycosaminoglycans (GAGs) are covalently attached. There are data demonstrating that ECM molecules are important in determining tissue viscoelastic behavior (17, 27, 28, 49). Al-Jamal et al. (3) have recently shown in lung parenchymal strips that digestion of the GAG component of PGs results in alterations in the viscoelastic properties of the lung tissue (3).

A number of studies (7, 34, 48) have examined the effects of mechanical forces on various types of ECM. In the lung, Xu et al. (46, 47) have documented that cyclic mechanical strain of mixed fetal lung cell cultures resulted in a reduction of message for both collagen I and biglycan and an increase in collagen IV. Another study (6) in fetal lung fibroblast cells subjected to mechanical strain reported somewhat different findings. There was an increase in collagen I mRNA and total collagen expression when the cells were grown on either laminin or elastin but not on fibronectin (6). Nonetheless, these studies all reported a response to mechanical strain. In vivo, excessive mechanical strain of the lung may occur during mechanical ventilation, which is known to cause or augment lung injury (10, 13). The mechanism of “ventilator-induced lung injury” is not completely understood but may be attributed in part to the effects of excessive airway pressure and alveolar overdistension (12, 43, 45). Atelectrauma, i.e., injury related to the shear forces generated by repetitive opening and closing of lung units, may also contribute (41). Data have been reported in animal models that show that altered ventilation regimens involving high airway pressures or large lung inflations result in increased message for the ECM proteins, procollagen, laminin, and fibronectin (5, 35).

Lung injury caused by excessive mechanical stimulation is characterized by alterations in lung tissue mechanical behavior. Webb and Tierney (45) originally showed that dynamic compliance decreased substan-
tially in rats ventilated with high inflation pressures and 0 positive end-expiratory pressure (PEEP). Tremblay et al. (43) documented changes in static lung compliance in isolated rat lungs ventilated for 2 h with high tidal volumes and 0 PEEP. Less is known about the changes in the resistive properties of lung tissues. One potential mechanism to explain the alteration in tissue properties may include mechanical stimulation-induced alterations in ECM proteins. We hypothesized that the changes observed in lung tissue mechanics with excessive ventilation might, in part, be explained by the effects of altered ventilation on ECM components.

To address this question, we performed the following experiment. We subjected anesthetized rats to different regimens of mechanical ventilation over a 2-h time period. Dynamic lung tissue mechanics were measured with a small-animal ventilator that has the capacity to deliver a multifrequency signal to the lungs and thereby allow measurement of complex impedance (40). After physiological measurements were completed, the lung tissue was examined by immunohistochemistry, and protein extraction and Western blotting were done to determine whether changes occurred in ECM PGs coincident with the changes in tissue mechanical behavior.

MATERIALS AND METHODS

Animal preparation. Adult specific pathogen-free male Sprague-Dawley rats (Charles River, St. Constant, PQ) weighing 150–200 g were used in this study. The animals were anesthetized with pentobarbital sodium (30 mg/kg ip) and xylazine (14 mg/kg ip). The carotid artery was cannulated with PE-90 tubing. After 1 h of ventilation, a sample of 300 μl was drawn for arterial pH and gas tension measurements. The body temperature was monitored and maintained at 35–36°C with a heating pad. A tracheotomy was performed, and a 14-gauge metal cannula was inserted into the trachea. The cannula was connected to a computer-controlled small-animal ventilator (40). The rats were mechanically ventilated at 90 breaths/min with a tidal volume (VT) of 8 ml/kg. The animals were then paralyzed with pancuronium bromide (0.8 mg/kg iv); supplemental pentobarbital sodium (PaCO2) between 35 and 45 Torr. A small amount of supplemental pentobarbital sodium (30 mg/kg ip) and PEEP [0 (PEEP 0) or 1.5 (PEEP 15) cmH2O]. Animals were ventilated at a frequency of 90 breaths/min for 2 h.

Experimental Animals formulated by the Canadian Council for Animal Care in compliance with the Guide to the Care and Use of Experimental Animals formulated by the Canadian Council of Animal Care, and protocols were approved by an institutional ethics committee.

Measurement of respiratory mechanics. Complex impedance was measured every 30 min by applying a volume oscillation to the trachea for an 18-s period during interruption of mechanical ventilation. A signal was composed of 12 sinusoids of frequencies ranging from 0.25 to 9.125 Hz. The amplitudes of the sinusoids decreased hyperbolically with frequency. The signal had a peak-to-peak volume of 0.5 ml. Piston displacement and cylinder pressure were measured and stored on a computer for calculation of total respiratory impedance (Z). The Z measurement of the total respiratory system (Zrs) was calculated by fitting the constant-phase model (19)

\[
Z_{rs} = R_{aw} + 2\pi f I_{aw} + (G - 2\pi f H)/(2\pi f) \tag{1}
\]

where Raw is airway resistance, Iaw is airway inertance, G and H are tissue resistance and elastance, respectively, and f is frequency. Hysteresivity (η) was calculated as

\[
\eta = G/H. \tag{2}
\]

After physiological measurements, the thorax was opened, the animal was exsanguinated, and the lungs were removed en bloc. The right lung was clamped, placed in 10 mM sodium acetate (pH 6.0), and frozen at −80°C for PG extraction. The left lung was filled with Histofix (1.5 ml), covered with optimum cutting temperature compound, and frozen while inflated. Blocks of tissue were cut into 5-μm sections with a cryostat.

PG extraction. Lung tissue samples frozen in buffer were sectioned into 20-μm slices with a cryostat. PGs were extracted with 4 M guanidium hydrochloride (10 ml/g tissue) and 1% (vol/vol) Triton X-100 in 100 mM sodium acetate (pH 5.8) containing 10 mM 6-aminohexanoic acid, 5 mM benzamidine hydrochloride, 1 mM phenylmethylsulfonyl fluoride, 10 mM EDTA, and 10 mM N-ethylmaleimide for 48 h at 4°C. The extracts were then subjected to dialysis against 10 mM sodium acetate (pH 6.0) containing protease inhibitors for 24 h at 4°C followed by dialysis against distilled deionized water. The extracts were precipitated overnight with 9% (vol/vol) ethanol at −20°C. The samples were precipitated by centrifugation at 12,000 rpm for 40 min at 4°C (10 μl of PG extract was used for the agarose-polyacrylamide composite gels and 20 μl of PG extract was used for SDS-PAGE). Samples to be analyzed by agarose-polyacrylamide composite gels were resuspended in 0.5% SDS-DETO and mixed 1:1 (vol/vol) with 50% (wt/wt) sucrose in 10 mM Tris-HCl (pH 6.8) and 0.05% bromophenol blue. Samples to be analyzed by SDS-PAGE were resuspended in 100 mM sodium acetate-Tris-HCl (pH 7.3) followed by digestion with chondroitinase ABC (0.2 U/ml extract) for 4 h at 37°C and then boiled for 5 min. Electrophoresis sample buffer was added [0.125 M Tris-HCl, pH 6.8, 20% (vol/vol) glycerol, 4% (wt/vol) SDS, 10% (vol/vol) β-mercaptoethanol, and 0.05% (wt/vol) bromophenol blue] and then denatured by boiling for 5 min before being loaded for SDS-PAGE.

Gel electrophoresis. Composite gels (1.5 mm thick) containing 0.6% agarose and 1.2% polyacrylamide in Tris-acetate buffer (10 mM, pH 6.8) containing 0.25 mM sodium sulfate were used for the separation of the large PGs, versican, and the large basement membrane (BM) heparan sulfate (HS) PG under associative conditions. The electrophoresis was performed at 70 V for the first hour and then at 150 V until the dye had migrated 5 cm. Electrophoresis was performed at 4°C. For the separation of the small PGs biglycan and fibromodulin, 10% SDS-PAGE was used, and electrophoresis was performed in 0.025 M Tris-0.192 M glycine buffer containing 0.1% (vol/vol) SDS.

After the electrophoretic separation, the samples were transferred with 0.025 M Tris-0.192 M glycine buffer containing 20% (vol/vol) methanol onto Hybond enhanced chemiluminescence (ECL)-nitrocellulose membranes with the wet blotting unit (Bio-Rad, Mississauga, ON) at 30 V overnight at 4°C.
The membranes were blocked with 5% ECL blocking solution in Tris-buffered saline (TBS), pH 7.4, containing 0.05% (vol/vol) Tween 20 and 0.05% sodium azide for 2 h at room temperature and then incubated with primary antibodies diluted in TBS-Tween 20 (TBS-T) for 1 h at room temperature. The primary antibodies were mouse anti-human at a 1:1,000 dilution for versican (12C5; Hybridgetm Bank, Iowa City, IA), mouse anti-EHS large BM HSPG at a 1:1,000 dilution for HSPG (C17; Hybridgetm Bank), and rabbit anti-human, which cross-reacted with rat PGs, at dilutions of 1:1,000 and 1:500 for biglycan and fibromodulin, respectively. A biotinylated secondary antibody (1:1,000 dilution in TBS-T) was added followed by a 1-h incubation with a 1:1,000 streptavidin-horseradish peroxidase complex in TBS-T. The signal was visualized with the ECL assay (Amersham Pharmacia, Montreal, PQ), and densitometry analysis was performed with the FluorChem FC 800 system (Alpha Innotech, San Leandro, CA).

Immunohistochemistry. Immunohistochemical staining was performed on 5-μm-thick lung sections. The slides were blocked by incubating with universal blocking solution, Montreal, PQ). Sodium acetate, Fast Red salt, sodium chloride, Triton X-100, 6-aminohexanoic acid, benzamidine hydrochloride, N-ethylmaleimide, SDS, agarose, acrylamide, N,N’-methylene-bis-acrylamide, Tween 20, sodium azide, glycin, sucrose, bromphenol blue, and chondroitinase ABC from Proteus vulgaris (protease free) were purchased from Sigma-Aldrich Canada (Oakville, ON). Hybond ECL-nitrocellulose membranes, ECL blocking solution, Optimum cutting temperature compound was obtained from the fluorophore (vol/vol) Tween 20 and 0.05% sodium azide for 2 h at room temperature. The slides were then rinsed with TBS (0.5 M Tris, pH 7.6, and 1.5 M NaCl) and incubated with the primary antibody. After a wash with TBS, the tissue was incubated with a 1:200 biotin-labeled swine anti-rabbit or goat anti-mouse IgG in TBS for 1 h, rinsed with TBS, and then further incubated with 1:100 alkaline phosphatase-conjugated avidin in TBS for 1 h. After further washes, the sections were developed with Fast Red salt (1 mg/ml) in alkaline phosphatase substrate for 10 min at room temperature. The sections were counterstained with Harris hematoxylin for 1 min and then washed with water. The sections were covered with a thin layer of crystal mount and dried in the oven at 37°C overnight.

Chemicals. Pentobarbital sodium was purchased from Health Canada (MTC Pharmaceuticals, Cambridge, ON), and xylazine was from CDMV (Bayer, Etobicoke, ON). Heparin was obtained from Organon Teknika (Toronto, ON). Pancuronium bromide was obtained from Sabex (Boucherville, PQ). Sodium acetate, Fast Red salt, sodium chloride, Tris, Harris hematoxylin, guanidinium hydrochloride, Triton X-100, 6-aminohexanoic acid, benzamidine hydrochloride, phenylmethylsulfonyl fluoride, N-ethylmaleimide, SDS, agarose, acrylamide, N,N’-methylene-bis-acrylamide, Tween 20, sodium azide, glycin, sucrose, bromphenol blue, and chondroitinase ABC from Proteus vulgaris (protease free) were purchased from Sigma-Aldrich Canada (Oakville, ON). His-toxin was purchased from Polysciences (Warrington, PA). Optimum cutting temperature compound was obtained from Fisher Scientific (Montreal, PQ). Universal blocking solution, biotin-labeled swine anti-rabbit IgG, goat anti-mouse IgG, alkaline phosphatase-conjugated avidin, and crystal mount were purchased from DAKO Diagnostics (Mississauga, ON). Rabbit anti-human biglycan and fibromodulin were gifts from Dr. Peter J. Roughley (Shriner’s Hospital, Montreal, PQ). Hybond ECL-nitrocellulose membranes, ECL blocking agent, streptavidin-alkaline phosphatase complex, ECL de-veloping solution, and ECL Hyperfilm were purchased from Pharmacia Amersham (Montreal, PQ).

Statistical analysis. The data were analyzed with SysStat for Windows, version 7.0.1 (SPSS, Chicago IL). A general linear model (repeated measures) was used to estimate and test the interaction between the changes in Raw, G, and H with VT and PEEP during the period of ventilation. Kruskal-Wallis one-way analysis of variance (ANOVA) was used to compare Raw, G, and H among the various groups at each time point separately. PG densitometry measurements among groups were analyzed with two-way ANOVA followed by Kruskal-Wallis one-way ANOVA for comparison between groups. Significance was set at P < 0.05. Results are means ± SE.

RESULTS

The animals ventilated at VT30PEEP0 were excluded from all the analyses because this regimen did not result in adequate gas exchange (pH = 7.07 ± 0.01; PaCO2 = 98.7 ± 2.3 Torr). In all other groups, relatively normal arterial blood gases were obtained (PaCO2 = 41.5 ± 5.3 Torr; PaO2 = 132.3 ± 11.5 Torr; pH = 7.38 ± 0.04).

Respiratory mechanics. Changes in Raw, G, and H over the 2-h period of ventilation are shown in Figs. 1–3. There was no change in Raw after 1.5 and 2 h of ventilation in the VT30PEEP group (Fig. 1). Ventilation at VT30PEEP0 resulted in a significant increase in G after 2 h (P < 0.05; Fig. 2). H was significantly increased in both the VT30PEEP1.5 (P < 0.05) and VT30PEEP0 (P < 0.05) groups after 2 h. Furthermore, a significant increase in H was observed in the VT30PEEP group after 1.5 h of ventilation (P < 0.05; Fig. 3). η did not change throughout the ventilation period in any of the five groups (data not shown).

PG expression by Western analysis. Expression of versican was significantly increased in animals subjected to VT30PEEP0 compared with those subjected to VT30PEEP0.
VT8PEEP1.5 (P < 0.01) and VT20PEEP0 (P < 0.05; Fig. 4). The expression of BM HSPG (Fig. 5) was significantly increased after ventilation at VT30PEEP0 compared with ventilation at VT8PEEP1.5 (P < 0.05). Densitometric analysis of biglycan expression revealed a significant increase after 2 h of mechanical ventilation in tissues from animals ventilated at VT30PEEP0 compared with animals ventilated at VT8PEEP1.5 (P < 0.05), VT30PEEP1.5 (P = 0.05), and VT20PEEP0 (P < 0.05; Fig. 6). The small keratan sulfate PG fibromodulin showed no change with any of the different ventilation regimens (data not shown).

**PG localization by immunohistochemistry.** To determine the site of increased protein expression, we examined the tissues using immunohistochemistry. Versican staining was detected at very low levels in the VT8PEEP1.5 group. Staining was observed mainly in airway and blood vessel walls and, to a lesser extent, in the alveolar wall (Fig. 7A). In animals ventilated at VT20PEEP1.5 or VT20PEEP0, there appeared to be a slight increase in staining for versican but no change in its localization (photomicrographs not shown). In tissues from animals ventilated at VT30PEEP1.5 and VT30PEEP0, an increase in the intensity of versican staining was observed in all regions, i.e., in the alveolar, airway, and blood vessel walls. In addition, in lung tissues from animals ventilated at VT30PEEP0, versican was also detected within the alveolar space (Fig. 7B). A similar pattern of staining was observed with the different ventilation regimens for BM HSPG (Fig. 7, C and D). Biglycan was detected only in very small amounts within the airway and blood vessel walls in animals ventilated with all regimens except VT30PEEP0. With this regimen, biglycan staining increased; however, staining continued to be limited to these structures. Negative control tissues incubated with the goat anti-mouse Ig secondary antibody from animals ventilated at VT30PEEP1.5 and VT30PEEP0 are shown in Fig. 7, E and F.

**DISCUSSION**

In this study, we examined the effects of different ventilatory regimens on dynamic respiratory mechanics and ECM composition. Ventilation with a large VT and 0 PEEP (VT30PEEP0) led to significant increases in both G and H. The change in lung mechanics coincided with a significant increase in the expression and altered distribution of the large chondroitin sulfate PG versican and the large BM HSPG. Furthermore, expression of the small chondroitin sulfate PG biglycan increased, although no alteration in the pattern of distribution was observed.

We ventilated animals with the chosen regimens, i.e., 2 h at VT of 8, 20 or 30 ml/kg and PEEP of 0 or 1.5 cmH2O based on a previous study (11) that showed that, in the rat, ventilation with such strategies was sufficient to induce lung injury. Changes in G were...
significant only at VT30PEEP0. Changes in H were significant at both VT30PEEP1.5 and VT30PEEP0, although the increase in H was much greater in the latter instance.

One of the limitations of our measurement system is that the mechanical properties of the chest wall were not separated from the mechanical properties of the respiratory system as a whole. In rats, the chest wall contributes significantly to the resistive and elastic properties of the respiratory system in a volume-dependent fashion (21). Raw reflects airway mechanical properties. However, we do not think that chest wall mechanics were altered as a consequence of overdistension because there is no evidence in the literature documenting such a link. In fact, the chest wall has been reported to limit overdistension (20).

Both volutrauma and atelectrauma may have contributed to the observed mechanical changes. Volutrauma (11, 43) describes the ability of volume distension to induce lung injury. Atelectrauma (41) describes the effect of ventilation at PEEP0. In this case, alveolar units are continuously recruited and derecruited, and the repetitive opening and closing of these units leads to extensive damage due to the shear forces generated. The alterations in G and H could be, in part, due to edema. Barnas et al. (4) have reported that both tissue resistance and H were increased after the induction of severe edema with oleic acid in dogs. Although we did not directly examine the tissues for evidence of edema, it is reasonable to assume that some fluid leak occurred. Microvascular permeability is likely altered because of alveolar-capillary membrane stress failure (16). Surfactant inactivation could also be a contributing factor (12, 45).
A further potential mechanism to explain the observed changes in tissue mechanics is an alteration in the composition of the ECM. Recent studies have described alterations in the matrix components in response to abnormal ventilation regimens. Berg et al. (5) described increased procollagen and fibronectin mRNAs after ventilation with high levels of PEEP. Parker et al. (35) found that ventilation at high peak airway pressures led to increased mRNA expression of these factors as well as of laminin B, another ECM glycoprotein. In vitro studies by Xu et al. (46, 47) and others (6, 32) have demonstrated the effects of mechanical strain on lung cells in culture. These studies have shown that mechanical strain affects ECM synthesis and secretion. Furthermore, preliminary data from our laboratory (2) have shown that in vitro mechanical strain of cultured human lung fibroblasts selectively alters PG synthesis.

Al-Jamal et al. (3) have recently reported that PGs affect lung tissue viscoelastic behavior. Selective degradation of the GAG side chains of these molecules with chondroitinase ABC or heparitinase I resulted in altered viscoelastic properties of excised parenchymal lung strips. Ebihara et al. (15) have also demonstrated in a bleomycin model of lung fibrosis that changes in tissue viscoelasticity correlated with alterations in the PG biglycan and occurred well before the changes in lung collagen were evident.

The mechanisms whereby PGs and GAGs in the matrix affect tissue viscoelasticity are not known, but several possibilities exist. The hydrophilic nature of these molecules attracts ions and fluid into the matrix, which affects tissue turgor and thereby potentially influences tissue viscoelastic behavior (38). PG molecules such as decorin and biglycan are known to coat individual collagen fibers (24). Mijailovich et al. (31) have proposed that energy dissipation occurs not at the molecular level within collagen or elastin but rather at the level of fiber-fiber contact and by the shearing of GAGs that provide the lubricating film between adjacent fibers. The ability of these molecules to influence the biomechanical behavior of the system may lie in their being “hardwired” to other matrix molecules and/or the cell membrane; they therefore could play a role in stress transfer between the ECM and the cellular component (23).

In the present study, mechanical ventilation at VT30PEEP0, induced a significant increase in both G and H that was accompanied by a significant increase in the three major subclasses of PGs. The first major subclass, the hyalectins, is represented by versican, the large aggregating chondroitin sulfate PG that has been demonstrated to be present in the lung (37). Together with the scaffoldlike structure of hyaluronan, to which versican is bound, this meshwork formation has the ability to regulate water movement in the tissues and hence affect its turgor (29). Versican expression not only increased in quantity, as seen from the Western analysis, but also exhibited altered distribution. Although versican was seen in the alveolar, airway, and blood vessel walls in control lungs, in animals ventilated at VT30PEEP0, there was prominent staining in the alveolar space. One could postulate that the presence in the alveolar space of versican molecules with their capacity to bind water could contribute to the edema known to occur under these experimental conditions.

The large BM HSPG, which is likely to be the 400- to 450-kDa (core protein size) perlecan and is a member of the second major subclass, BM PGs, exhibited similar changes in both protein expression and distribution. HSPG is known to represent a barrier to the passage of cationic macromolecules across the BM (24). It interacts with other matrix components such as laminin, fibronectin, integrin, and collagen IV as well as with the cellular component, thus maintaining the integrity of the tissue by regulating stress transfer (24). Our
Fig. 7. Immunohistochemical staining for versican (A and B) and BM HSPG (C and D). E and F: negative control tissues incubated with goat anti-mouse Ig secondary antibodies. A, C, and E: representative sections from animals ventilated at VT8PEEP1.5. B, D, and F: representative sections from animals ventilated at VT30PEEP0. Staining for both versican and BM HSPG increased after ventilation at VT30PEEP0. In addition, protein expression was not only evident in alveolar, airway, and blood vessel walls but was also observed in the alveolar space (arrowheads). Original magnification, ×200.
finding of the presence of BM HSPG is in agreement with a recent report by Gronski et al. (18), who described perlecan in the bronchoalveolar lavage fluid of closed-chest mice subjected to excessive mechanical ventilation. The increased levels of HSPG detected are consistent with ventilation-induced damage to the BM. An altered BM may also have permitted leak of excess versican into the alveolar space.

The significance of the additional bands shown in the Western blots for versican and BM HSPG at VT30PEEP0 may reflect changes in the glycosylation patterns of these molecules, which, in turn, would affect their mobility (9, 42). Alternately, it may reflect a different core protein size, which can occur as a result of alternative splicing induced by excessive ventilation. The central domain of versican is composed of GAG-α and GAG-β, which are subject to alternative splicing. To date, four isoforms have been recognized in mammalian tissues (50). HSPGs are a heterogeneous family that has been reported to occur in multiple forms (8, 39).

We also examined the effects of excessive mechanical ventilation on biglycan and fibromodulin expression, two members of the small, leucine-rich family of PGs. Biglycan was increased at VT30PEEP0; however, the distribution of this molecule remained limited to the airway and blood vessel walls. Biglycan has been suggested to play a role in ECM organization through its ability to bind both matrix collagens I and VI (26). This molecule may also play a role in stress transfer. The expression of fibromodulin was unchanged after 2 h of mechanical ventilation with any of the ventilation regimens. Fibromodulin is known to affect collagen fibrillogenesis (25). It is possible that the duration of mechanical ventilation in our study was insufficient to see an increase in a molecule primarily involved with the remodeling process.

The ability of the cell to respond to mechanical strain may be explained by the hypothesis that cells are hard-wired via the cytoskeleton to the ECM (23). We postulated that on application of excessive mechanical load, the lung would respond by altering the composition of the load-bearing structures, i.e., the ECM. Indeed, a number of studies have examined the effects of mechanical forces of various types on ECM remodeling. Studies in cartilage (22, 44) have documented an increase in message for collagen and the aggregating PG aggrecan in response to cyclic compression. The aggrecan response was detected as early as 1 h (7). An in vivo study in hamsters (34) has shown that animals subjected to a sedentary lifestyle have decreased cartilage PG and GAG content. Robbins et al. (36) have also demonstrated that in tendons, cyclic mechanical stimulation resulted in an increase in the PGs aggrecan, biglycan, and versican and collagen I mRNA. A few studies have investigated this phenomenon in the lung. As reported above, there are published data (32, 46, 47) demonstrating the effect of cyclic mechanical strain on matrix production by lung tissue cells. Data by Al-Jamal et al. (2) in adult human lung fibroblasts show a direct effect on PG production.

The mechanism of the changes in PGs may be related to increases in transcription factors that are known to be elevated in response to mechanical strain. Mechanical forces have been reported to increase the transcription factors cAMP response element (CRE), Sp1, and nuclear factor (NF)-κB in human umbilical vein endothelial cells and transforming growth factor (TGF)-β in vascular smooth muscle cells (14, 30, 33). As to the level of regulation, the promoter of versican has a CRE and Sp1- and TGF-β-responsive elements. The perlecan promoter has TGF-β- and NF-κB-responsive elements that increase expression (24). Therefore, we can speculate that the increase in transcription factors could be the cause of the observed increase in PGs. Al-Jamal et al. (1, 2) have recently reported preliminary data on the effect of mechanical strain on versican expression and mRNA levels in vitro in human fibroblasts. They were able to demonstrate that mechanical strain increased both versican protein expression and mRNA.

Why the lung responds to mechanical ventilation by upregulating PG expression is a matter of conjecture. One hypothesis that we favor is that the increased secretion of PGs represents an “adaptive response.” PGs affect the viscoelastic behavior of the lung tissues. They are critical in the transmission of stress between the ECM, the cellular membrane, and the cytoskeleton. Hence altered PG secretion may represent a potential feedback mechanism whereby the system can adjust to altered mechanical requirements such as those imposed by excessive mechanical ventilation.

In conclusion, we have demonstrated that excessive mechanical ventilation results in altered lung tissue mechanics, coincident with increases in the PG molecules that comprise the ground substance of the ECM. We hypothesize that the changes in the ECM represent an autoregulatory response by the lung tissue cells to altered mechanical requirements. The precise mechanism driving this adaptive response warrants further study.

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