Phosphotyrosine phosphatase and tyrosine kinase inhibition modulate airway pressure-induced lung injury

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Parker, James C., Claire L. Ivey, and Allan Tucker. Phosphotyrosine phosphatase and tyrosine kinase inhibition modulate airway pressure-induced lung injury. J. Appl. Physiol. 85(5): 1753–1761, 1998.—We determined whether drugs which modulate the state of protein tyrosine phosphorylation could alter the threshold for high airway pressure-induced microvascular injury in isolated perfused rat lungs. Lungs were ventilated for successive 30-min periods with peak inflation pressures (PIP) of 7, 20, 30, and 35 cmH2O followed by measurement of the capillary filtration coefficient (Kfc), a sensitive index of hydraulic conductance. In untreated control lungs, Kfc increased by 1.3- and 3.3-fold relative to baseline (7 cmH2O PIP) after ventilation with 30 and 35 cmH2O PIP. However, in lungs treated with 100 µM phenylarsine oxide (a phosphotyrosine phosphatase inhibitor), Kfc increased by 4.7- and 16.4-fold relative to baseline at these PIP values. In lungs treated with 50 µM genistein (a tyrosine kinase inhibitor), Kfc increased significantly only at 35 cmH2O PIP, and the three groups were significantly different from each other. Thus phosphotyrosine phosphatase inhibition increased the susceptibility of rat lungs to high-PIP injury, and tyrosine kinase inhibition attenuated the injury relative to the high-PIP control lungs.

RECENT EXPERIMENTAL STUDIES have confirmed the long-standing suspicion of clinicians that the high airway pressures (Paw) used to maintain adequate oxygenation of patients with acute respiratory distress syndrome (ARDS) may augment the capillary leak of fluid and protein (23). In addition, the presence of an inflammatory lung disease may increase the susceptibility of the lung to mechanical injury by high ventilation pressures. Woodring (39) observed the presence of interstitial emphysema in the lungs of 88% of ARDS patients ventilated with peak inflation pressures (PIP) of 40 cmH2O or greater. In experimental animal studies, Hernandez et al. (14) and Dreyfuss et al. (7) found significant lung injury after ventilation with normally innocuous levels of PIP in the presence of minimal preexisting injury. These studies suggest that cellular changes after such injury may weaken parenchymal tissues and reduce their tolerance to mechanical stresses. In fact, the clinical application of very low tidal volume ventilation in ARDS patients has resulted in reduced mortality and morbidity in some studies (15).

The increased microvascular permeability to fluid and proteins induced by the increased mechanical stress of high vascular and Paw has generally been attributed to a passive failure of the vascular structures. Shirley et al. (32) proposed that the increased lymph and protein flow from peripheral capillaries subjected to high venous pressures was produced by the "stretched-pore phenomenon" caused by hydrostatic pressure forcing open the endothelial junctions. More recently, West et al. (38) proposed that "stress failure" of the alveolar capillary basement membrane resulted in increased capillary leak and hemorrhage at high vascular pressures. However, the endothelium and epithelium are generally considered to be the major barriers which restrict transcapillary fluid movement (35), and recent evidence from our laboratory (24, 25) suggests that microvascular permeability increases induced by mechanical stress may have a significant active cellular component. Parker and Ivey (24) reported that infusion of isoproterenol to increase intracellular cAMP concentrations significantly attenuated the increase in capillary filtration coefficient (Kfc) induced by high venous pressures in isolated rat lungs. In addition, infusion of gadolinium, to block stretch-activated cation channels in endothelium, prevented increases in Kfc after high-PIP ventilation in isolated rat lungs (24). These studies suggest an active ATP- and Ca2+-dependent contraction of cytoskeletal fibrils in endothelial cells after mechanical injury similar to that observed in endothelial monolayers, single capillaries, and isolated lungs in response to soluble mediators and ischemia (16, 19, 22).

A major determinant of the restrictive properties of endothelial and epithelial layers is the integrity of the cell-cell and cell-matrix adhesions which maintain continuity of the layers. The plasma-filled blebs observed, with the use of a microscope, between epithelial and endothelial cells and basement membrane after high-Paw ventilation in experimental animals suggest that release of cell-matrix adhesions may contribute to the increase in microvascular permeability accompanying mechanical injury (6). The number and strength of the cell adhesions that bind cells to the underlying interstitial matrix are greatly influenced by the state of tyrosine phosphorylation of focal adhesion proteins (40). Although the sequence of phosphorylation events involved in formation and remodeling of focal adhesions is incompletely understood, an increased tyrosine phosphorylation of focal adhesion proteins is associated with release of focal adhesions and increased cell motility, whereas both phosphorylation and dephosphorylation of tyrosine appear to be involved in remodeling adhesion sites (21). Release of focal adhesions that tether endothelial cells to the basement membrane...
near the margins of any intercellular openings would enhance margin retraction and vascular permeability.

The purpose of the present study was to determine whether the susceptibility of rat lungs to injury by high PIP could be altered by inhibition of phosphotyrosine phosphatase or tyrosine kinase. These cellular enzymes are involved in tyrosine phosphorylation and dephosphorylation of a number of intracellular proteins, including those in the focal adhesion complexes (4). Genistein is a relatively nonselective tyrosine kinase inhibitor, so it would reduce the level of protein tyrosine phosphorylation (4, 21). Phosphatase or tyrosine kinase. These cellular en-

METHODS

Isolated Rat Lung Preparation

The isolated rat lung preparation and its relationship to in situ lung injury and species differences have been previously described (11, 23, 25). Briefly, male Charles River CD rats weighing between 199 and 320 g were anesthe-
tized with an intraperitoneal injection of pentobarbital (65 mg/kg), the trachea was cannulated, and the rats were ventilated with 20% O2-5% CO2-75% N2 by using a Harvard rodent ventilator (model 683; South Natick, MA) with a tidal volume of 2.5 ml and a positive end-expiratory pressure (PEEP) of 3 cmH2O at 40 breaths/min. This tidal volume resulted in a nominal PIP of 6-7 cmH2O. Mean airway pressure (MAP) was calculated by using MAP = (PIP – PEEP)/3 + PEEP. The chest was opened, and 300 U sodium heparin were injected into the right vena. The pulmonary artery and left atrium were then cannulated, and the heart and lungs were excised en bloc and suspended from a force transducer. Lungs were perfused with 5% bovine serum albumin in Kreb’s bicarbonate buffer (37°C) at 6 ml·min

was obtained, the venous reservoir was raised to obtain the desired Ppv. For baseline Kfc measurements, Ppv was increased to 15 cmH2O and maintained for 20 min. Capillary pressure (Ppc) was measured by using the double occlusion pressure at baseline and at increased Ppv and the increase in capillary filtration pressure (ΔPpc) was the change in Ppc between vascular pressure states (35). The rate of weight gain (in g/min) was averaged over the last 2 min of the lung weight-gain curve (ΔWt-20) at increased Ppv and used to calculate Kfc by

\[ K_{fc} = \frac{\Delta W_{t-20}}{\Delta P_{pc}} \]

where \( t \) is time and \( \Delta W \) is change in weight. All Kfc values were normalized to 100 g PLW, which was based on body weight (BW) according to

\[ PLW = 0.0053 BW - 0.48 \]

and calculated as milliliters per minute per cmH2O per 100 grams by assuming a specific gravity of 1.0 for filtered fluid. Per fusate flow (Q) for each experiment was calculated from the PLW and set at 6 ml·min\(^{-1}\)·g\(^{-1}\) PLW. RT was calculated from Ppa and Ppv by using

\[ RT = \frac{(Ppa - Ppv)}{Q} \]

Hb Assay and Tissue Volumes

Lung samples were homogenized in distilled H2O (3.33 ml/g tissue) and centrifuged for 1 h at 15,000 rpm; the supernatant was retained. Total Hb (Hbtot) was measured in perfusate (b) and tissue supernatant (s) by using the cyanomet-hemoglobin method, and absorbance was read at 540 nm (6). Samples of perfusate and tissue homogenate (h) were stained with hematoxylin and eosin and viewed with light microscopy.

Experimental Protocols

High-PIP control group (n = 6). These rat lungs were isolated and prepared as described above. The general time course of Paw and Ppv increases are shown in Fig. 1. After a baseline period of 30 min, a baseline Kfc measurement was performed with a nominal PIP of 7 cmH2O. Then the lungs were ventilated for 30-min periods with 20 cmH2O PIP and 3 cmH2O PEEP, with 30 cmH2O PIP and 3 cmH2O PEEP, and with 35 cmH2O PIP and 8 cmH2O PEEP. The latter combination of Paw produced a consistent three- to fourfold increase in Kfc in this preparation. Each ventilation period was followed by a Kfc measurement for 20 min. After perfusion was stopped, the lungs were weighed and sectioned.

High-PIP genistein group (n = 6). These rat lungs were isolated and prepared as described above. After a baseline period of 30 min, a baseline Kfc was performed with a PIP
of ~7 cmH$_2$O. Genistein (50 µM) was added to the venous reservoir, and the protocol shown in Fig. 1 and described above was performed. After perfusion was stopped, the lung was weighed and sectioned.

High-PIP PAO group (n = 6). These rat lungs were isolated and prepared as described above. After a baseline period of 30 min, a baseline $K_{fc}$ measurement was performed with a PIP of ~7 cmH$_2$O. PAO (100 µM) was added to the venous reservoir, and the protocol shown in Fig. 1 and described above was performed. After perfusion was stopped, the lung was weighed and sectioned.

Low-PIP PAO group (n = 6). These rat lungs were isolated and prepared as described above. After a baseline period of 30 min, a baseline $K_{fc}$ measurement was performed with a PIP of ~7 cmH$_2$O. PAO (100 µM) was added to the venous reservoir, and the nominal baseline vascular pressures and PIP were maintained throughout the experiment. $K_{fc}$ was measured after each 30-min period as in the high-PIP protocol. After perfusion was stopped, the lung was weighed and sectioned.

Statistics

All values are expressed as means ± SE unless otherwise stated. Data group means were compared within and between groups by using an ANOVA with repeated measures and a Newman-Keuls posttest on CRUNCH4 statistical software and a Gateway 2000 digital computer. A two-way ANOVA was performed to establish a significant (P < 0.05) interaction between drug treatments and PIP or time before the Newman-Keuls test was applied. A logarithmic transformation of data was used to normalize the data, decrease skew, and equalize group variances before the ANOVA. A difference between means was determined to be significant at P < 0.05.

RESULTS

$K_{fc}$

Figure 2 shows $K_{fc}$ as a function of PIP in the high-PIP control, high-PIP genistein, and high-PIP phenylarsine oxide (PAO) groups. Values are means ± SE; n = 6. *P < 0.05 vs. baseline within same group. #P < 0.05 vs. all previous states within the same group. §P < 0.05 vs. all other groups at same PIP. †P < 0.05 vs. high-PIP genistein group at same PIP.

The high-PIP PAO group, by 2.1-fold at 30 cmH$_2$O PIP and 4.5-fold at 35 cmH$_2$O PIP in the high-PIP control group, and by 4.5-fold at 35 cmH$_2$O PIP in the high-PIP genistein group. $K_{fc}$ was significantly greater in the high-PIP PAO group than in all other groups at 30 cmH$_2$O PIP and 35 cmH$_2$O PIP. In addition, $K_{fc}$ for the high-PIP control group was significantly greater (2.2-fold) than that for the high-PIP genistein group at 30 cmH$_2$O PIP. Figure 3 is a plot of $K_{fc}$ as a function of time in the high-PIP PAO and the low-PIP PAO groups and
shows the effect of high-PIP mechanical stress in the presence of PAO. $K_t$ in the low-PIP group did not change significantly at any time period and was significantly less than that for the high-PIP PAO group at every time except the baseline period.

**Vascular Pressure and Paw**

Mean vascular pressure and Paw for the four groups are summarized in Table 1. There were no differences in Paw between high-PIP groups, but PIP was increased significantly and MAP approximately doubled after each increase in PIP. Vascular pressures shown in the table are those present after the high-PIP ventilation periods, when PIP was returned to baseline before measurement of $K_t$, and these vascular pressures were used to calculate the Rt. Increases in Rt at baseline Paw indicate the vascular responses to injury or edema formation rather than mechanical compression of alveolar vessels by high distending Paw (23, 35). Figure 4 shows Rt as a function of PIP in the high-PIP control, high-PIP genistein, and high-PIP PAO groups. There were no significant differences in vascular pressures, Paw, or Rt between groups at 7 and 20 cmH2O PIP. Rt was significantly increased from baseline by 1.7-fold at 35 cmH2O PIP and by 3.6-fold at 35 cmH2O PIP in the high-PIP PAO group. Rt was significantly increased from all lower PIP values at 35 cmH2O PIP in all three groups, but differences between groups were not significant. Figure 5 shows Rt as a function of time in the high-PIP PAO and low-PIP PAO groups. Rt increased significantly from baseline in the high-PIP PAO group, as shown, but Rt did not increase significantly after any period in the low-PIP PAO group.

**Tissue Fluid Volumes**

Figure 6 shows the final lung weights and residual perfusate weights for the four groups. For the high-PIP PAO, high-PIP control, high-PIP genistein, and low-PIP PAO groups, the final lung weights were 236, 192, 158, and 115%, respectively, of the PLW. Weights of all high-PIP lungs were significantly higher than those of the low-PIP PAO group. The lungs of the high-PIP PAO group also gained significantly more weight than those of the high-PIP genistein group. Residual blood weight

**Table 1. Vascular and airway pressures at each peak inflation pressure state**

<table>
<thead>
<tr>
<th>PIP, cmH2O</th>
<th>PEEP, cmH2O</th>
<th>MAP, cmH2O</th>
<th>Ppa, cmH2O</th>
<th>Ppv, cmH2O</th>
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<tr>
<td><strong>High-PIP control</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>6.7±0.3</td>
<td>2.9±0.1</td>
<td>4.2±0.1</td>
<td>7.4±0.2</td>
<td>3.6±0.2</td>
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<tr>
<td>19.8±0.2*</td>
<td>2.5±0.2</td>
<td>8.3±0.2</td>
<td>7.3±0.2</td>
<td>3.5±0.2</td>
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<tr>
<td>30.0±0.4†</td>
<td>3.3±0.5</td>
<td>12.2±0.4</td>
<td>8.4±0.3</td>
<td>3.4±0.2</td>
</tr>
<tr>
<td>35.0±0.1‡</td>
<td>7.7±1.2†</td>
<td>16.8±0.8‡</td>
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<td>3.7±3.8‡</td>
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<td><strong>High-PIP genistein</strong></td>
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<tr>
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<td>16.9±0.5‡</td>
<td>12.0±1.2‡</td>
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<tr>
<td>20.0±0.0*</td>
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<tr>
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</tr>
<tr>
<td>35.2±0.3‡</td>
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<td>17.6±0.6‡</td>
<td>17.8±2.7‡</td>
<td>3.3±0.3‡</td>
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<tr>
<td><strong>Low-PIP PAO</strong></td>
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<td></td>
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<tr>
<td>7.4±0.2</td>
<td>2.9±0.2</td>
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<tr>
<td>7.9±0.4</td>
<td>2.0±0.0</td>
<td>4.0±0.1‡</td>
<td>7.8±0.7</td>
<td>3.9±0.1‡</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 rats in each group. PIP, peak inflation pressure; PEEP, positive end-expiratory pressure; MAP, mean airway pressure; Ppa, pulmonary arterial pressure; Ppv, pulmonary venous pressure during high-PIP ventilation; PAO, phenylarsine oxide. *P < 0.05 vs. baseline state; †P < 0.05 vs. preceding 2 pressure states; ‡P < 0.05 vs. all preceding pressure states.

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**Fig. 4.** Pulmonary vascular resistance as a function of PIP in high-PIP control, high-PIP genistein, and high-PIP PAO groups. Values are means ± SE; n = 6. *P < 0.05 vs. baseline within same group. †P < 0.05 vs. all previous states within same group.

**Fig. 5.** Pulmonary vascular resistance as a function of time in high-PIP PAO and low-PIP PAO group. Values are means ± SE; n = 6. *P < 0.05 vs. baseline within same group. †P < 0.05 vs. all previous states within same group. §P < 0.05 vs. all other groups at same PIP.
in the high-PIP PAO group was significantly greater than that in the low-PIP PAO group, and the blood weight-to-lung weight ratios were 0.31, 0.30, 0.28, and 0.17, respectively, for the low-PIP PAO, high-PIP genistein, high-PIP control, and high-PIP PAO groups. The residual blood fraction in the low-PIP PAO group was within the range for passively drained, uninjured lungs from several species (35). Although not specifically measured, only the high-PIP groups had residual tissue blood weights indicative of significant extravascular hemorrhage.

**Histology**

Figure 7 shows representative histological sections from the four groups of lungs. Each picture shows a bronchus (left) and an artery (right) magnified ×130. The lungs in the low-PIP PAO group (Fig. 7A) appeared unremarkable except for focal areas of slight perivascular edema. Stromal cells were clearly identified in edematous areas, and no intra-alveolar edema was observed. In the high-PIP genistein group lungs (Fig. 7B), a moderate amount of edema was observed; some vessels exhibited perivascular cuffs, and others showed no appreciable edema. Slight amounts of intra-alveolar edema were observed. The findings in the high-PIP
control group (Fig. 7C) varied from animal to animal and among regions within each lung. Perivascular cuffs ranged from moderate to prominent, with a minority of vessels showing perivascular hemorrhage. A variable degree of intra-alveolar edema was observed that involved from 10 to 50% of alveolar spaces. The high-PIP PAO group lungs (Fig. 7D) were characterized by moderate to prominent hemorrhagic perivascular edema cuffs around a majority of the vessels observed. Prominent edema cuffs surrounded those vessels without hemorrhage, and patchy intra-alveolar edema was observed. The histological observation of interstitial bleeding indicates that much of the increase in residual blood volume in the high-PIP groups was extravascular.

**DISCUSSION**

In previous studies on single capillaries and endothelial monolayers, inhibition of phosphotyrosine phosphatase has been observed to directly increase paracellular permeability, but the relationship of tyrosine phosphorylation and mechanical stress injury in intact lungs has not been previously described. Adamson (1) observed a rapidly reversible 6.5-fold increase in hydraulic conductance (Lp) in single frog capillaries after infusion of 30 μM PAO, and Vincent et al. (36) reported a decrease in transcellular electrical resistance across endothelial monolayers after inhibition of phosphotyrosine phosphatase by using either of the tyrosine phosphatase inhibitors (PAO or sodium orthovanadate) to increase the cellular levels of tyrosine-phosphorylated proteins. An increased tyrosine phosphorylation of the focal adhesion proteins, focal adhesion kinase (pp125FAK), and paxillin were observed, as were dissociation of the adherens junctions between cells and reorganization of the fibrin cytoskeleton (1, 36). PAO enhanced intercellular gap formation, paracellular permeability, and actin reorganization induced by either H₂O₂ or lipopolysaccharide (LPS) in both endothelial (2, 4) and epithelial monolayers (27). In addition, Staddon et al. (33) found that PAO decreased electrical resistance in both endothelial and epithelial monolayers and enhanced tyrosine phosphorylation of adherens junction β-catenin and tight junction ZO-1 in epithelial cells. Increased phosphorylation of β-catenin has been associated with release of cadherin binding between cells (17). Thus increased tyrosine phosphorylation due to phosphotyrosine phosphatase inhibition causes disintegration of occludens (cell-cell) and cell-matrix focal adhesions, with concomitant increases in paracellular permeability (36).

In the present study, there was no significant increase in $K_{fc}$ with time after infusion of PAO in the low-PIP PAO group, and there was no evidence of edema formation or interstitial hemorrhage in this group. This was surprising, because a lower dose induced permeability increases in frog capillaries (1). In contrast, there were dramatic increases in $K_{fc}$ in the high-PIP PAO group as a function of PIP, where $K_{fc}$ increased significantly to 5.1 and 11.9 times baseline at 30 and 35 cmH₂O PIP, respectively. These $K_{fc}$ increases were approximately twice the respective values in the high-PIP control group at these two PIP states. The higher lung weights and residual blood volumes, as well as histological evidence of edema and extravasated red cells in the interstitial spaces, correlated with the increases in $K_{fc}$ observed in the high-PIP groups. That is, all of the measured indicators of edema and injury were greatest for the high-PIP PAO group. The enhanced effects of high Paw on $K_{fc}$ suggest that tissue adhesions were weakened by PAO but remained sufficiently strong to maintain the structural integrity of the capillaries in the absence of additional mechanical stress. The increases in residual blood weight in high-PIP groups suggest that the mechanical stress induced a separation of vascular structures, such as the basement membrane, sufficient to allow passage of red blood cells. Some perivascular cuffs containing red blood cells were observed in the high-PIP control group, and edema cuffs without red blood cells were observed in the high-PIP PAO group, but red blood cell extravasation and edema were greater after PAO. Although altered cellular adheresiveness could account for the increased $K_{fc}$ in the high-PIP PAO group, the mechanism of PAO in enhancing red blood cell extravasation is unknown. A presumed increase in intracellular tyrosine phosphorylation induced by PAO infusion may have lowered the threshold for high PIP increases in $K_{fc}$ in these isolated rat lungs through a reduced strength of cell-cell and cell-matrix adhesions that could increase tissue susceptibility to mechanical injury.

An increased tyrosine phosphorylation of focal adhesion proteins is associated with increased cell motility, release of some adhesions, shape changes, and realignment of actin stress fibers (40). However, not all adhesion sites may be affected equally, and this fact could account for the absence of a significant increase in $K_{fc}$ in the low-PIP PAO group. Takagi and Saito (34) found that the highly elevated levels of tyrosine phosphorylation in fibroblasts caused by vanadate and PAO were accompanied by loss of β₁-integrin-mediated adhesion to collagen and laminin but not β₂-integrin adhesion to fibronectin and vitronectin. A reduced number of adhesion sites could be sufficient to maintain tissue integrity at low vascular and distending Paw but lead to cell separations at high Paw.

An altered cell adhesion may also account for the increased susceptibility to high-PIP lung injury in the presence of preexisting lung disease or injury in patients and experimental animals. Ventilation of patients for treatment of ARDS, gastric aspiration, or pneumonia results in high incidences of tissue air leaks at PIP that are well tolerated by uninjured lungs (23). Endotoxins and H₂O₂ increase both phosphotyrosine levels and endothelial monolayer permeability (2, 4, 27), and the lung injury of ARDS has a significant oxidant-mediated component (35). Hernandez et al. (14) found that ventilation of rabbit lungs with modest levels of PIP (24 cmH₂O) after infusion of subthreshold doses of oleic acid produced significant $K_{fc}$ increases, although neither insult alone increased vascular perme-
A reduced intracellular Ca$^{2+}$ may inhibit the activity of tyrosine kinases by genistein. This may inhibit the active tension generated by the cytoskeleton, which is crucial for the maintenance of focal adhesions after mechanical separation of cells.

Formation of focal adhesion of cells to the underlying extracellular matrix is a complex process, and the interaction of the various intracellular proteins and adhesion molecules with mechanical stress is incompletely understood. Oliver et al. (21) observed a dephosphorylation of pp125{$^c$}FAK, paxillin, and pp60{$^c$}src after mechanical detachment of cultured alveolar epithelial cells from underlying matrix. PAO inhibition of phosphotyrosine phosphatase prevented both protein dephosphorylation and cell reattachment, but inhibition of tyrosine kinase with genistein had no effect on reattachment. They postulated different sequences of protein phosphorylation for the initial formation and reattachment of focal adhesions after mechanical separation of cells.

The significantly lower $K_{fc}$ at 30 cmH$_2$O PIP in lungs treated with genistein compared with control lungs was surprising in view of studies showing a lack of effect of genistein on the rate of reattachment of epithelial cells mechanically removed from their substrate (21). However, initial formation of focal adhesions and cell spreading was reduced by genistein in endothelial cell cultures (29, 40). Genistein and other tyrosine kinase inhibitors prevented permeability increases in endothelial and epithelial monolayers exposed to H$_2$O$_2$ or endotoxin and reduced the increased levels of tyrosine phosphorylation of intercellular and cell-matrix adhesion proteins induced by these agents (2, 4, 27). Because the activities of both tyrosine kinase and protein tyrosine phosphatase were required for internalization of integrins by B cells (30), inhibition of tyrosine kinase may have complex effects on processing of adhesion proteins.

Other possible mechanisms for the attenuation of the $K_{fc}$ increase by genistein include an inhibition of actin depolymerization and cytoskeletal reorganization and a reduction in the influx of intracellular Ca$^{2+}$ in microvascular endothelial cells. Genistein prevented G-actin and gap formation in endothelial cells after endotoxin (4) and attenuated receptor-mediated Ca$^{2+}$ influx (3, 9). Inhibition of tyrosine kinases by genistein may inhibit the protein kinase C at higher doses than those used in the present study, and this could also reduce Ca$^{2+}$ influx (31). A reduced intracellular Ca$^{2+}$ would reduce the active tension generated by the cytoskeleton, which may retract the free edges of mechanically induced openings through the endothelial and epithelial layers in the capillary barrier (18, 22). Studies in single frog capillaries by Pegakis and Curry (22) have demonstrated the necessary coupling of increased endothelial intracellular Ca$^{2+}$ to receptor-mediated increases in permeability, in which an influx of extracellular Ca$^{2+}$ was required for an increase in $L_p$ (13). In their preparation, regional endothelial Ca$^{2+}$ spikes were always associated with macromolecular leak sites (22), but endothelial Ca$^{2+}$ transients could be uncoupled from the $L_p$ increase by increasing intracellular cAMP, thus indicating an active actin-myosin interaction involved in the permeability increase (12).

The most likely source of increased endothelial Ca$^{2+}$ during mechanical distention of lung capillaries by high Paw is the stretch-activated cation channels. In studies of endothelial monolayers stretched on a silicone membrane, Naruse and Sokabe (19) measured a peak in intracellular Ca$^{2+}$ which was abolished either by removal of Ca$^{2+}$ from the medium or by adding 10 µM gadolinium (Gd$^{3+}$) to block nonspecific stretch-activated cation channels. Recently, Parker et al. (25) infused Gd$^{3+}$ in isolated rat lungs subjected to the same high-PIP protocol described in the present study, and $K_{fc}$ did not increase above baseline even after ventilation with 35 cmH$_2$O PIP and 8 cmH$_2$O PEEP. Parker and Ivey (24) also reported a 64% reduction in the increase in $K_{fc}$ induced by high Ppv in the same rat lung preparation. Isoproterenol is thought to relax endothelial cells and to decrease microvascular permeability by increasing cAMP and by inhibition of myosin light chain kinase activity and subsequent contraction of actin-myosin filaments (16, 18). Those studies suggest an active, Ca$^{2+}$- and ATP-dependent cellular response to mechanical injury that is similar in many ways to that observed for receptor-mediated permeability increases in endothelial monolayer and intact capillaries. Therefore, inhibition by genistein of any kinase that reduced endothelial Ca$^{2+}$ influx would have dramatic effects on transvascular fluid conductance.

The results obtained in the present study and other recent studies suggest that revisions are warranted to the current hypotheses regarding the mechanism of mechanical stress-induced increases in vascular permeability. Shirley et al. (32) originally postulated a stretched pore effect of a large volume expansion in dogs based on increases in the lymph-to-plasma ratio of proteins as well as flow in thoracic and right lymph ducts. Subsequent experimental studies have confirmed that an increased pulmonary microvascular permeability is induced both at high Ppv (10, 28) and at alveolar overdistention with high volumes and pressures (10, 26). More recently, elegant electron microscopic studies by West and colleagues (see Refs. 5, 8, 10, and 38) and Neal and Michel (20) indicate that transcellular leakage of fluid actually occurs via newly formed transcellular openings through the endothelial and epithelial layers rather than through a passive widening of the intercellular junctions.

West et al. (38) have subsequently postulated a stress failure of the alveolar capillary basement membrane as
a mechanism for mechanical injury, because the tensile strength of the basement membrane is much greater than any cellular component in the alveolar capillaries (37). Although basement membrane rupture undoubtedly determines the amount of hemorrhage at very high pulmonary vascular pressures, the endothelial cellular layer is generally considered to be the major barrier to microvascular fluid filtration at pressures below the threshold for capillary rupture (35). The ability to modulate high-PIP injury by presumptive alterations in intercellular and cell-matrix adhesion in the present study, to attenuate high vascular pressure-induced increases in rat lung $K_w$ with isoproterenol (24), and to prevent high-PIP $K_w$ increases by blockade of stretch-activated cation channels (25) argues for an active participation of endothelium and other parenchymal cells in the permeability response to mechanical injury. We envision a Ca$^{2+}$- and ATP-dependent stretch-induced recoil of the margins of these transcellular openings in endothelial cells induced by stress, which is facilitated by cytoskeletal reorganization and release of cell-matrix adhesions. An active widening of the margins of these openings would then augment transcapillary fluid filtration. Such a mechanism could serve to release vascular pressure stress to preserve the interstitial scaffolding, so that cells could more readily reestablish an effective alveolar capillary barrier when the excessive stress is removed. These studies also suggest that the signaling pathways involved in the capillary leak response to mechanical injury may be amenable to pharmacological intervention.

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