Clara cell secretory protein and phospholipase A2 activity modulate acute ventilator-induced lung injury in mice

Sawako Yoshikawa, Takashige Miyahara, Susan D. Reynolds, Barry R. Stripp, Mirece Anghelescu, Fabien G. Eyal, and James C. Parker. Clara cell secretory protein and phospholipase A2 activity modulate acute ventilator-induced lung injury in mice. J Appl Physiol 98: 1264–1271, 2005. First published December 17, 2004; doi:10.1152/japplphysiol.01150.2004.—Lung vascular permeability is acutely increased by high-pressure and high-volume ventilation. To determine the roles of mechanically activated cytosolic PLA2 (cPLA2) and Clara cell secretory protein (CCSP), a modulator of cPLA2 activity, we compared lung injury with and without a PLA2 inhibitor in wild-type mice and CCSP-null mice (CCSP−/−) ventilated with high and low peak inflation pressures (PIP) for 2- or 4-h periods. After ventilation with high PIP, we observed significant increases in the bronchoalveolar lavage albumin concentrations, lung wet-to-dry weight ratios, and lung myeloperoxidase in both genotypes compared with unventilated controls and low-PIP ventilated mice. All injury variables except myeloperoxidase were significantly greater in the CCSP−/− mice relative to wild-type mice. Inhibition of cPLA2 in wild-type and CCSP−/− mice ventilated at high PIP for 4 h significantly reduced bronchoalveolar lavage albumin and total protein and lung wet-to-dry weight ratios compared with vehicle-treated mice of the same genotype. Membrane phospho-cPLA2 and cPLA2 activities were significantly elevated in lung homogenates of high-PIP ventilated mice of both genotypes but were significantly higher in the CCSP−/− mice relative to wild-type mice. Inhibition of cPLA2 significantly attenuated both the phospho-cPLA2 increase and increased cPLA2 activity due to high-PIP ventilation. We propose that mechanical activation of the cPLA2 pathway contributes to acute high PIP-induced lung injury and that CCSP may reduce this injury through inhibition of the cPLA2 pathway and reduction of proinflammatory products produced by this pathway.

capillary permeability; arachidonyl trifluoromethyl ketone; mechanical stress; knockout mice

VENTILATOR-INDUCED LUNG INJURY (VILI) has been recognized as a significant contributing factor to the morbidity and mortality of patients with acute respiratory distress syndrome (4). The role of inflammation in lung distention-induced permeability pulmonary edema is controversial. Some investigators advocate the view that inflammatory mediators initiate the increase in vascular permeability, whereas others propose that an acute increase in permeability helps initiate inflammation and propagation of injury (7, 37). Evidence from our laboratory supports the latter view because the increase in microvascular permeability occurred rapidly in both isolated and in situ lungs during lung overdistention by high tidal volumes or airway pressures (39, 40). Cytokine elevations were variable and observed later during VILI, and there were no acute lung permeability differences between wild-type and TNF dual-receptor knockout mice (8, 39). Although the factors that initiate the increased vascular permeability and propagate the inflammatory response remain poorly understood, rapidly responding mediators and signal pathways are likely involved. A pathway that rapidly responds to mechanical stress but has not previously been linked to VILI is the phospholipase A2 (PLA2) pathway. Activated PLA2 can have myriad proinflammatory effects by catalyzing the hydrolysis of phospholipids to form arachidonic acid and lysophospholipids (2), which are further metabolized to prostaglandins, leukotrienes, hydroxyeicosatetraenoic acids, or platelet-activating factor. Many of these products can increase vascular permeability, recruit neutrophils, and injure cells (26). PLA2 activity was increased in cultured endothelial cells subjected to mechanical stress via a p42 MAPK pathway, so overdistention of the lung would be expected to activate PLA2 (14, 30).

The Clara cell secretory protein (CCSP) is an endogenous modulator of lung inflammation that has not previously been investigated with respect to the etiology of mechanical injury. Because CCSP has an inhibitory effect on PLA2 activity, it is likely that this inhibition is a mechanism for its inflammation-suppressing action (5). This protein represents ~2–3% of the total protein content of human bronchoalveolar lavage (BAL) fluid (12). Mice deficient in CCSP had increased lung injury and inflammatory responses after exposure to oxidant stress or virus challenge (11, 17, 23), and lavage CCSP was found in higher levels in patients who survived acute respiratory distress syndrome than in those who did not (6). Because CCSP inhibits both PLA2 and transglutaminase, which amplifies the activity of PLA2, the injurious effects of the multiple downstream mediators would be reduced (2, 25).

In the present study, we investigated the contributions of PLA2 and CCSP to the lung permeability response to VILI using an inhibitor of PLA2 in wild-type and CCSP knockout mice. Lavage albumin concentration and wet-to-dry lung weight (W/D) ratios were used to assess vascular permeability and pulmonary edema formation, respectively, and lung myeloperoxidase and lavage cell counts were used to track the inflammatory response after ventilation with high and low peak inflation pressures (PIP). CCSP-deficient (CCSP−/−) mice were more susceptible to high-PIP ventilation injury than genetically matched controls, and inhibition of cytosolic PLA2...
(cPLA₂) attenuated injury in both wild-type and CCSP⁺/⁻ mice. Injury was also correlated with an increased phosphorylation state and enzymatic activity of membrane-localized cPLA₂.

METHODS

Animal Preparation

All animal protocols were approved by the Institutional Animal Care and Use Committee at the University of South Alabama. Male C57BL/6 strain mice, which were CCSP⁺/⁻, and strain-matched wild-type control mice were obtained after weaning from breeding colonies at the University of Pittsburgh and maintained as specific pathogen-free in-house colonies at the University of South Alabama animal facility until used for experiments.

Experiments were conducted on 50 mice, 24 wild-type and 26 CCSP⁺/⁻ mice, which were divided into 10 groups. Mice ranging in weight from 27.8 to 33.2 g (mean of 29.0 ± 0.5 g) were anesthetized with pentobarbital (65 mg/kg). A cannula was inserted through a tracheostomy into the trachea, and mice were ventilated using a rodent ventilator (Harvard, model 683, South Natick, MA) as previously described (40). Airway pressure was measured using a Cobe pressure transducer (Lakewood, CO), and an electrocardiogram was monitored using a polygraph (Grass, model 70, Quincy, MA).

Experimental Protocols

All protocols and analyses were similar for each group except for the ventilation periods of either 2 or 4 h. Inhibition of cPLA₂ was obtained by intraperitoneal injection of 20 mg/kg arachidonoyl trifluoromethyl ketone (ATK; Cayman Chemical, Ann Arbor, MI) in the drug.

Two-hour ventilation protocols. Wild-type mice were divided into three experimental groups: (1) a high-PIP group (n = 6), (2) a low-PIP group (n = 3), and (3) a control group (n = 5). Groups of CCSP⁺/⁻ mice consisted of (1) a high-PIP group (n = 6) and (2) a control group (n = 5).

Four-hour ventilation protocols. Wild-type mice were divided into (1) a high-PIP vehicle-treated group (n = 5), (2) a high-PIP group treated with ATK (n = 5), and (3) a low-PIP group (n = 5). Groups of CCSP⁺/⁻ mice consisted of (1) a high-PIP vehicle-treated group (n = 5) and (2) a high-PIP group treated with ATK (n = 5).

The high-PIP wild-type and CCSP⁺/⁻ groups were ventilated with 50 cmH₂O of PIP and 2.5 cmH₂O positive end-expiratory pressure at 17 breaths/min for either 2 or 4 h. The low-PIP groups of wild-type mice were ventilated at a PIP of 15 cmH₂O for 2 or 4 h at 120 breaths/min with a tidal volume of ~0.29 ml. A low-PIP ventilation group was not done for the CCSP⁺/⁻ mice because sufficient mice were not available. The control groups of wild-type and CCSP⁺/⁻ mice were intubated and ventilated for a brief period with a PIP of 15 cmH₂O at 120 breaths/min to allow blood sampling. The low ventilatory rate in the high-PIP groups was necessary to maintain pH and blood gases comparable to other groups. A delivered tidal volume of ~1 ml was calculated from the airway pressures and lung compliances for the high-PIP groups (35). Tidal volume was adjusted as necessary to maintain PIP constant throughout the experiment. Blood was sampled by cardiac puncture for gas analysis after ventilation.

BAL

The right lung was lavaged four times with 0.5 ml of phosphate buffered saline. Total cell counts were measured using a hemocytometer, and albumin concentrations were measured with an ELIZA (Bethyl, Montgomery, TX). Total protein was measured using a modified Bradford assay. Differential cell counts were performed using Wright’s stain.

Myeloperoxidase Activity

After BAL collection, the right middle lung lobe was removed and homogenized in 0.5% hexadecyl trimethylammonium bromide in phosphate buffered saline. Samples were centrifuged, and supernatant was reacted with tetramethylbenzidine (1.6 mM) and 0.1 mM H₂O₂ for 6 min. Myeloperoxidase (MPO) activity was expressed as a change in absorbency at 450 nm.

W/D Ratios

The left lung was weighed for wet weight and desiccated at 80°C for 1 wk to obtain the dry weight for the W/D ratio. The W/D ratios in the 4-h groups were corrected for the dry weight of alveolar total protein by assuming the same protein leak in both lungs such that

Corrected W/D = W[(D − (V₂BAL × C₂P) ]

where W and D are wet and dry weights, respectively, of the left lung and V₂BAL and C₂P are volume and total protein concentrations, respectively, of the BAL fluid.

Immunoblotting

The right lower lung lobe was frozen in liquid nitrogen. The lungs were homogenized in buffer and centrifuged at 100,000 g for 1 h. The cytosolic supernatant was separated, and the membrane pellet was resuspended in buffer. Fractions were mixed with Laemmli buffer, and equal amounts of proteins (50 µg) were subjected to 7.5% SDS-PAGE. Protein was transferred to nitrocellulose membranes and probed with anti-cPLA₂ antibody and anti-phospho cPLA₂ (Ser505) antibody (Cell Signaling, Beverly, MA). Bound antibody was detected by a chemiluminescent detection system kit (Cell Signaling), quantified by using a computer scanner, and density calculated using Sigmagel software.

PLA₂ Activity

Activity of calcium-dependent cPLA₂ was determined in lung homogenates using a cPLA₂ assay kit obtained from Cayman Chemical (Ann Arbor, MI). Reaction of cPLA₂ with arachidonoyl thiosphorylcholine was indicated using Ellman’s reagent, and the color was measured using a plate reader at 414 nm.

Statistics

All values are expressed as means ± SE. Comparison between groups was performed using a two-way ANOVA following Fishers least significant difference analysis. Significant differences were determined where P < 0.05.

RESULTS

Tidal Volume

Tidal volume per gram of body weight required to produce an initial PIP of 50 cmH₂O was not significantly different between the two genotypes (wild type: 0.090 ± 0.002 ml/g; CCSP⁺/⁻: 0.092 ± 0.002 ml/g), indicating similar lung mechanics between groups. Over a 2-h period of high-PIP ventilation at a fixed tidal volume, PIP decreased by 3.7 ± 0.2 cmH₂O in wild-type mice and by 3.8 ± 0.3 cmH₂O in CCSP⁺/⁻ mice, suggesting a small increase in compliance during prolonged overdistention. Terminal arterial blood gases are summarized in Table 1. Because only one sample could be analyzed after the experimental period, it was only possible to correct ventilation parameters for the next experiment and not during an experiment. These blood gases suggest a moderate amount of hyperventilation in all mechanically ventilated conditions.
groups. The high Po2 values obtained after lung injury are surprising but may relate to supplemental oxygen administered during experiments, where indicated, and possibly to gas exchange during the open-chest cardiac-puncture procedure.

**Lavage Albumin and Total Protein Concentrations**

*Two-hour ventilation studies.* Albumin concentration in lavage was used as an indicator of increased vascular permeability after lung injury induced by ventilation (Fig. 1). No significant difference was observed between the lavage albumin concentrations of wild-type and CCSP−/− control mice. High-PIP ventilation induced a significant 4.2-fold increase in lavage albumin concentration in wild-type mice and a significant 6.7-fold increase in CCSP−/− mice. The lavage albumin concentration was significantly higher in CCSP−/− mice ventilated with high-PIP compared with wild-type mice.

*Four-hour ventilation studies.* Figure 2 indicates the lavage albumin and total protein concentrations in the 4-h ventilation studies. Figure 2A shows that the lavage albumin concentrations were significantly increased in all high-PIP groups compared with 4-h ventilation with low PIP. After 4 h of ventilation, lavage albumin was 3.9 times the low-PIP group value in the high-PIP wild-type mice and 5.0 times the low-PIP group value in the high-PIP CCSP−/− mice. However, lavage albumin was significantly 30% greater in the high-PIP CCSP−/− group than in the high-PIP wild-type group. Compared with their respective 2-h high-PIP ventilation groups, the lavage albumin concentrations were 31% greater at 4 than at 2 h in the high-PIP wild-type mice and 29% greater at 4 h compared with 2 h in the CCSP−/− group. Pretreatment with the PLA2 inhibitor ATk resulted in significant decreases in lavage albumin by 38% in the high-PIP CCSP−/− mice and 36% in the high-PIP wild-type mice compared with their respective untreated high-PIP groups.

The lavage total protein concentrations in the same groups reflected the similar trends observed for lavage albumin as shown in Fig. 2B. Lavage total protein was significantly increased in all high-PIP groups compared with the low-PIP groups, and ATk treatment significantly attenuated this increase by 33% in the high-PIP CCSP−/− group and 36% in the high-PIP wild-type group compared with their respective untreated high-PIP groups. However, there were no statistical differences between the wild-type and CCSP−/− groups.
Two-hour ventilation studies. W/D ratio was used as an indicator of edema formation induced by ventilation (Fig. 3). No significant difference was observed in the W/D ratios from wild-type control and CCSP−/− control mice. High-PIP ventilation for 2 h induced significant increases in the W/D ratio to 18% in wild-type mice and 39% in CCSP−/− mice compared with control values. W/D ratios in CCSP−/− mice were significantly greater by 16% than those of wild-type mice.

Four-hour ventilation studies. Figure 4 shows that high-PIP ventilation for 4 h induced significant increases in the W/D ratios in all high-PIP groups compared with the low-PIP group. W/D ratios corrected for alveolar total protein were 35% greater in high-PIP wild-type mice and 50% greater in high-PIP CCSP−/− mice compared with the low-PIP group. The mean W/D ratio in high-PIP CCSP−/− mice was greater by 11% than that of high-PIP wild-type mice, and this difference was statistically significant. Pretreatment with ATK resulted in significantly lower W/D ratios after high-PIP ventilation in both wild-type (15% lower) and CCSP−/− mice (13% lower), but both ATK-treated and untreated CCSP−/− high-PIP groups had significantly greater W/D ratios than measured in the ATK-treated high-PIP wild-type group.

BAL Total Cell Counts and MPO Activities

Total cell count and MPO activity for groups ventilated for 2 and 4 h are summarized in Table 2. There were no differences in lavage total cell count between wild-type and CCSP−/− control mice. The cell counts in all 2- and 4-h high-PIP ventilation groups were significantly reduced relative to unventilated controls, but the 2- and 4-h low-PIP groups were not different from controls. There were no statistical differences in lavage total cell counts between the genotypes after ventilation with high PIP for 2 or 4 h or in the leukocyte percentages. Differential counts indicated 68.0 ± 0.6% of macrophages and 32.0 ± 0.6% of lymphocytes in wild-type mice and 67.3 ± 0.8% of macrophages and 32.7 ± 0.8% of lymphocytes in CCSP−/− mice after 2 h of high-PIP ventilation. Numerous red cells were present in the lavage of the high-PIP groups but were not present in the low-PIP controls after both 2 and 4 h of high-PIP ventilation.

Table 2 indicates the major differences in MPO activity related to time and pressure rather than genotype and treatment. No significant differences were observed in MPO activity between control groups of wild-type and CCSP−/− mice. High-PIP ventilation for 2 h induced significant increases in MPO activity in both wild-type and CCSP−/− mice compared with control groups, but the 4-h high-PIP groups had significantly higher MPO activities than the 2-h high-PIP groups and the 4-h low-PIP group. There appeared to be a trend toward a higher MPO in the high-PIP CCSP−/− groups because mean MPO activity was 12% greater in the CCSP−/− group after 2 h of high PIP and 26% higher after 4 h compared with the wild-type mice, but there were no statistical differences between these genotypes. Pretreatment with ATK also had no statistical effect on MPO activity after 4 h of high-PIP ventilation.

Level of Membrane Localized Phospho-cPLA2

The levels of total cPLA2 expression were not significantly different between the control groups of wild-type and CCSP−/− mice, and 2 h of high-PIP ventilation did not significantly change the total cPLA2 levels in either group (Fig. 5A).

Table 2. BAL total cell counts and myeloperoxidase activity in wild-type and CCSP−/− mice after 2- and 4-h ventilation periods with high and low PIP

<table>
<thead>
<tr>
<th>Ventilation Time, h</th>
<th>Total Cell Counts, 10⁴/mm³</th>
<th>Myeloperoxidase, OD g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control wild type</td>
<td>0.25</td>
<td>3.89 ± 0.89</td>
</tr>
<tr>
<td>Control CCSP−/−</td>
<td>0.25</td>
<td>3.80 ± 0.81</td>
</tr>
<tr>
<td>Low PIP wild type</td>
<td>2</td>
<td>3.41 ± 1.10</td>
</tr>
<tr>
<td>High PIP wild type</td>
<td>2</td>
<td>2.08 ± 0.21*</td>
</tr>
<tr>
<td>High PIP CCSP−/−</td>
<td>2</td>
<td>2.83 ± 0.63*</td>
</tr>
<tr>
<td>Low PIP wild type vehicle</td>
<td>4</td>
<td>4.94 ± 0.28</td>
</tr>
<tr>
<td>High PIP wild type vehicle</td>
<td>4</td>
<td>4.94 ± 0.28</td>
</tr>
<tr>
<td>High PIP wild type ATK</td>
<td>4</td>
<td>3.17 ± 0.14‡</td>
</tr>
<tr>
<td>High PIP CCSP−/− + ATK</td>
<td>4</td>
<td>2.37 ± 0.21*</td>
</tr>
</tbody>
</table>

Values are means ± SE. BAL, bronchoalveolar lavage. *P < 0.05 vs. unventilated control group of both genotypes. †P < 0.05 vs. 2-h high-PIP groups and 4-h low-PIP group. ‡P = 0.07–0.16 vs. other 2- and 4-h high-PIP ventilation groups. NA, not available.
or the ratio of the membrane fraction of cPLA2 to total cPLA2 in either phenotype. Protein bands shown are from the same gel exposed for 15 s for total cPLA2 levels and 60 s for phospho-cPLA2 to obtain densities in an appropriate range for quantitation. In contrast, the mean (n = 4) phospho-cPLA2 in the membrane fraction (Fig. 5B) and the ratio of phospho-cPLA2 to total cPLA2 were significantly increased in wild-type (2.1-fold) and CCSP−/− mice (2.7-fold) after 2 h of high-PIP ventilation normalized for that of wild-type control mice. Immunoblots from 3 independent experiments were averaged to obtain mean density values. *P < 0.05 vs. control. #P < 0.05 vs. wild-type high-PIP group.

Membrane phospho-cPLA2 was also significantly increased after 4 h of high-PIP ventilation in lung homogenates in wild-type mice compared with 4 h of low-PIP ventilation shown by differences in the band densities in Fig. 6A. Figure 6B summarizes mean densities on membrane phospho-cPLA2 in four experiments and indicates a 2.2-fold higher density after high-PIP ventilation.

An analysis of tissue cPLA2 activity (n = 4) indicated a significant increase in cPLA2 activity after 4 h of high-PIP ventilation compared with 4 h of low-PIP ventilation in wild-type mice (Fig. 7). After 4 h of high-PIP ventilation, cPLA2 activity in vehicle-treated mice was 2.6-fold that in the low-PIP ventilation group. Pretreatment with ATK significantly attenuated this increase in activity so that cPLA2 activity was 1.9-fold higher than that in the low-PIP group and significantly less than that in the vehicle-treated high-PIP group. The cPLA2 activity in the CCSP−/− mice after 4 h of high-PIP ventilation was also significantly increased over the wild-type low-PIP group, and this increase in activity was significantly attenuated by pretreatment with ATK. Ventilation with high PIP resulted in a cPLA2 activity in the CCSP−/− mice that was 45% higher than that in the wild-type mice, a difference that approached statistical significance (P < 0.056). The high-PIP CCSP−/− group activity was also significantly greater than the high-PIP wild-type group treated with ATK.
**DISCUSSION**

The novel aspects of our findings were that CCSP deficiency in mice increased the susceptibility of the lungs to acute VILI, whereas inhibition of PLA2 attenuated lung vascular permeability and edema in both CCSP-/- and wild-type mice after 2 and 4 h of high-PIP ventilation. Increases in microvascular permeability and edema formation were evidenced by an increase in lavage albumin concentration and W/D ratios in the CCSP-/- mice compared with wild-type CCSP+/+ mice. Albumin was considered a more specific marker of permeability than total proteins because total protein measurements may also reflect proteins secreted by lung cells. There were also significantly greater amounts of phosphorylated cPLA2 and a greater cPLA2 activity in the lungs of CCSP-/- mice compared with CCSP+/+ mice after high-PIP ventilation but reduced amounts of phosphorylated cPLA2 and reduced cPLA2 activity in groups treated with ATK, the PLA2 inhibitor. MPO activity increased significantly in relation to ventilation time and pressure and was greater in the 2- and 4-h high-PIP groups relative to low-PIP ventilation groups and unventilated controls, but PLA2 inhibition or absence of CCSP did not significantly alter MPO activity. However, lavage total cell counts actually decreased in the high-PIP groups.

The time course for lung injury and inflammation during VILI has previously been studied in both mice and rats. Yoshikawa et al. (39) previously observed time- and pressure-dependent increases in lung transcapillary protein fluxes and edema in intact mice. A size-selective transcapillary exchange of CCSP, albumin, and IgG increased as the PIP and ventilation periods were increased. The present study supports these observations since lavage albumin, total protein, and W/D ratios were greater in the high-PIP groups compared with low-PIP groups and in the 4-h compared with 2-h high-PIP groups for both wild-type and CCSP-/- mice. Because there were no differences in normalized tidal volumes required to produce a PIP of 50 cmH2O, it is unlikely that differences in the baseline mechanics of the lungs accounted for the differences between genotypes. In previous studies, no differences in production of surfactant mRNA or surfactant were observed between the lungs of CCSP-/- and wild-type mice (15, 34).

The relationship of the vascular permeability lesion to the inflammatory response after VILI has generated considerable controversy because both perfused and unperfused isolated lung preparations readily produce inflammatory cytokines such as TNF-α, whereas ventilated intact animals have much less of an inflammatory response (7, 36). In intact mice ventilated at high PIP, the increase in lung vascular permeability preceded the appearance of inflammatory cytokines in BAL by over 1 h, and there were no differences in the BAL albumin and W/D ratios between TNF-α-null mice and wild-type mice (39). The rapid initial permeability response suggests that rapidly formed mediators or rapid signal events, such as response elements of the PLA2 pathway, may help initiate this acute response. The greater albumin and W/D ratio responses observed in the CCSP-/- compared with CCSP+/+ mice suggest that CCSP has a role in partially reducing both the increase in lung permeability and inflammatory responses after mechanical damage in intact animals, as has previously been demonstrated for infectious and chemical lung insults (11, 17, 23).

The ability of CCSP to inhibit PLA2 activity is the best studied chemical action of CCSP (5, 22). In cultured-cell experiments, CCSP was shown to inhibit secretory PLA2 by binding calcium (2), a cofactor of secretory PLA2 activation, or phosphatidylcholine, a substrate of PLA2. CCSP also lowered the cPLA2 activity in fibroblasts stimulated by interleukin-1β (21). However, C57BL/6 mice have an inbred deficiency in secretory PLA2 but not cPLA2, which can be activated by increased intracellular Ca2+ or phosphorylation by activated MAPK (ERK, JNK, p38) (20, 41). Both increased Ca2+ and MAPK activation occur in stretched endothelial cells and in endothelial and epithelial cells in intact lungs during distention (3, 28). Likewise, PLA2 activation has been reported after mechanical stress in endothelial cells in culture (30) and in intact blood-perfused lungs (19). On activation, cPLA2 translocates to the perinuclear and intercellular junctional membranes (9, 20), but maximal activation requires phosphorylation of cPLA2 (29).

The evidence for a causative link between CCSP and PLA2 activity and VILI is as follows. First, BAL albumin and W/D ratios were significantly higher after 2 and 4 h of high-PIP ventilation in CCSP-/- mice compared with wild-type mice, which is indicative of a greater vascular permeability increase in CCSP-/- mice. Second, high-PIP ventilation in the CCSP-/- mice resulted in significantly higher phosphorylation of cPLA2 after 2 h, and the cPLA2 activity was also marginally greater in the CCSP-/- mice (45%; P < 0.056). Third, inhibition of cPLA2 activity with ATK significantly reduced indexes of injury in both CCSP-/- and wild-type mice after 4 h of high-PIP ventilation. The reduction in BAL albumin concentration of 92 mg/ml for CCSP-/- mice vs. 67 mg/ml in wild-type mice and the greater reduction in cPLA2 activity in CCSP-/- mice of 50 vs. 28% in wild-type mice suggest that the cPLA2 activity increase may have contributed more to the injury in CCSP-/- mice than that of wild-type mice. These effects strongly suggest that CCSP modulates mechanical lung injury primarily via its effect on PLA2 activity. However, ATK pretreatment reduced lavage albumin and edema by only about one-third in high-PIP ventilation groups, so additional factors besides cPLA2 products would be expected to contribute to the mechanical injury. In addition, the significantly higher edema formation, which persisted in CCSP-/- mice after ATK treatment relative to wild-type mice, also suggests that CCSP may have additional modulating effects in addition to inhibition of cPLA2 activity.

Inhibition of cPLA2 could attenuate VILI by reducing numerous downstream products. cPLA2 is a proinflammatory enzyme that hydrolyzes membrane phospholipids to free arachidonic acid, lysophosphatidic acid, and platelet-activating factor (20, 26). Arachidonic acid is further metabolized by cyclo-oxygenase, lipoxygenase, and P450 pathways to inflammatory mediators such as prostaglandins, thromboxanes, leukotrienes, and epoxyeicosatrienoic acids (41). Candidate mediators for the increased lung microvascular permeability are direct actions of platelet-activating factor (10) or lysophosphatidic acid (13), prostaglandin E2 induction of proteases (33), leukotriene B4 attraction of neutrophils (38), or P450 metabolites (1). Recently, Alvarez et al. (1) showed a significant permeability increase in isolated rat lungs in response to infusion of the epoxyeicosatrienoic acid P450 derivatives, 5,6-epoxyeicosatrienoic acid and 14,15-epoxyeicosatrienoic.
acid. Because CCSP inhibited arachidonic acid release in an adenocarcinoma cell line (31) and ablation of the cPLA2 gene in mice inhibited lung injury due to sepsis, zymosan infusion, or acid aspiration (27), one might speculate that the presence of CCSP would alter the inflammatory response to high-PIP ventilation by altering cPLA2 activity.

Neutrophil accumulation in the lung is one of the pathological features of VILI, and neutrophil accumulation increases progressively with time during high-PIP ventilation (16, 18). High-PIP ventilation increased MPO activity in a time- and pressure-dependent manner in the present study. MPO increased significantly by 75% in both genotypes after 2 h of ventilation compared with unventilated controls and by ~35-fold after 4 h of high-PIP ventilation. MPO after 4 h of low-PIP ventilation was also 65% greater than in unventilated controls. Although mean MPO in the CCSP−/− mice was 17% higher than that of the CCSP+/+ mice after 2 h of high-PIP ventilation and 35% greater after 4 h, the difference was not significant between the two genotypes. Consequently, a difference in neutrophil accumulation is unlikely to explain the augmented permeability response in the CCSP−/− mice. However, simple neutrophil numbers may not accurately represent the complex interactions of neutrophils with endothelial cells because neutrophil activation was also increased by mechanical ventilation (24). Activation of neutrophils could be increased by several arachidonic acid derivatives. However, previous studies in isolated perfused rat lungs ventilated with high PIP suggest a permeability response that occurs much more rapidly than that attributed to neutrophil recruitment (39, 41).

Paradoxically, the lavage total cell counts actually decreased significantly, suggesting that the MPO measurements represented marginating or interstitial neutrophils. The decrease in total cell count in lavage may represent the movement of activated macrophages into the interstitial spaces as previously described (16). Quinn et al (32) ventilated rats for 2 h at high tidal volumes (20 ml/kg) and observed significant increases in lung W/D ratios, but lavage macropage inflammatory protein-2 and neutrophil counts did not change. Lung MPO also was not elevated after 2 h of high-PIP ventilation in their study but increased significantly after an additional 6-h wait. In a previous study using our ventilated mouse preparation, lavage cell counts also were not increased significantly after only 2 h of high-PIP ventilation in the CCSP+/+ mice (40). The increased MPO suggests that neutrophils may adhere to lung vessels within 2 h of high-PIP ventilation without massive immigration into the alveolar spaces.

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GRANTS

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