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SURFACTANT DYSFUNCTION IN LUNG CONTUSION WITH AND WITHOUT SUPERIMPOSED GASTRIC ASPIRATION IN A RAT MODEL

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Abstract

This study investigates surfactant dysfunction in rats with lung contusion (LC) induced by blunt chest trauma. Rats at 24 h postcontusion had a decreased percent content of large surfactant aggregates in cell-free bronchoalveolar lavage (BAL) and altered large-aggregate composition with decreased phosphatidylcholine (PC), increased lyso-PC, and increased protein compared with uninjured controls. The surface activity of large aggregates on a pulsating bubble surfactometer was also severely impaired at 24 h postcontusion. Decreases in large surfactant aggregate content and surface activity were improved, but still apparent, at 48 and 72 h postcontusion compared with uninjured control rats and returned to normal by 96 h postcontusion. The functional importance of surfactant abnormalities in LC injury was documented in pilot studies showing that exogenous surfactant replacement at 24 h postcontusion improved inflation/deflation lung volumes. Additional experiments investigated a clinically relevant combination of LC plus gastric aspiration (combined acid and small gastric food particles) and found reductions in large surfactant aggregates in BAL similar to those for LC. However, rats given LC + combined acid and small gastric food particles versus LC had more severe surfactant dysfunction based on decreases in surface activity and alterations in large aggregate composition. Combined data for all animal groups had strong statistical correlations between surfactant dysfunction (increased minimum surface tension, decreased large aggregates in BAL, decreased aggregate PC, and increased aggregate lyso-PC) and the severity of inflammatory lung injury (increased total protein, albumin, protein/phospholipid ratio, neutrophils, and erythrocytes in BAL plus increased whole lung myeloperoxidase activity). These results show that surfactant dysfunction is important in the pathophysiology of LC with or without concurrent

gastric aspiration and provides a rationale for surfactant replacement therapy in these prevalent clinical conditions.

Keywords

Lung surfactant; surfactant dysfunction; lung contusion; aspiration; acute lung injury

INTRODUCTION

Blunt chest trauma is involved in nearly one third of acute trauma admissions to the hospital (1–3), and lung contusion (LC) is an important and prevalent problem in the care of the critically ill trauma victim. Lung contusion is frequently complicated by witnessed or unwitnessed gastric aspiration at the time of trauma or by pneumonia during subsequent hospitalization. Lung contusion is also an independent risk factor for the development of acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) (1–3). These latter clinical syndromes reflect severe acute inflammatory lung injury and have high mortality and morbidity despite significant improvements in cardiorespiratory intensive care during the past several decades (4). When LC leads to hypoxemia severe enough to qualify as ALI/ARDS, the prognostic and economic impacts are significant. In a 2004 study of trauma patients, the incremental hospital cost per patient with ALI or ARDS (\$36,713 or \$59,633, respectively) was much higher than for patients without ALI/ARDS (\$24,715) (5).

The pathophysiology of LC includes hypoxemia, hypercarbia, increased work of breathing, and decreased pulmonary volumes in association with ventilation/perfusion mismatching, edema, and segmental lung damage (1,6,7). However, little previous work has addressed lung surfactant dysfunction in this clinically important condition. Because of the essential physiological roles of lung surfactant, its dysfunction may be an important determinant in the severity and progression of clinical LC. The present study seeks to improve understanding about mechanisms relating to surfactant dysfunction in bilateral LC induced by blunt chest trauma in a rat model developed in our laboratory (8,9). Surfactant dysfunction is investigated at 24 to 96 h postinjury based on the content and surface activity of large surfactant aggregates in bronchoalveolar lavage (BAL). The lipid and protein composition of large surfactant aggregates is also determined at 24 h postinjury, when surface activity deficits are most pronounced. Indices of surfactant dysfunction are also correlated statistically with the severity of lung injury based on concentrations of total protein, albumin, and erythrocytes in BAL, and with inflammation as reflected by the numbers of polymorphonuclear leukocytes (PMNs) in BAL and on whole lung levels of myeloperoxidase (MPO). The functional importance of surfactant dysfunction in LC is further assessed in pilot studies of exogenous surfactant therapy with the clinical surfactant drug Infasurf at 24 h postcontusion.

In addition to studying isolated bilateral LC, experiments were performed to address surfactant dysfunction when contusion is complicated by concurrent gastric aspiration. The aspirate used in these latter experiments was a mixture of hydrochloric acid plus small gastric particles (combined acid and small gastric particles; CASP), which we have previously shown to cause severe inflammatory lung injury in rodent models (10–15). Gastric aspiration is a major direct pulmonary injury cause of ALI/ARDS and occurs frequently in patients who have a brief loss of consciousness or risk factors such as food or alcohol intake at, or near, the time of trauma (16,17). Many cases of gastric aspiration are unwitnessed or unreported, and gastric aspiration pneumonitis in the clinical setting is typically a diagnosis of exclusion in patients without other known direct causes of respiratory compromise (16,17). The scenario of concomitant pulmonary contusion plus gastric aspiration in chest trauma patients may be significantly

underappreciated because affected patients have a readily apparent presumptive cause of lung injury from trauma alone.

Studies in this article test several hypotheses relating to surfactant dysfunction in LC with or without concurrent CASP aspiration. Specific hypotheses are (1) that significant surfactant dysfunction occurs in LC injury, including decreased large aggregate content, reduced surface activity, and altered aggregate lipid/protein composition; (2) that exogenous surfactant therapy at 24 h post-LC injury will improve lung mechanics and/or reduce albumin leakage; (3) that the severity of surfactant dysfunction is exacerbated in LC + CASP compared with LC alone; and (4) that the magnitude of surfactant dysfunction for all animal groups will correlate with the severity of permeability injury based on albumin and total protein in BAL and with pulmonary inflammation based on leukocytes in BAL and whole lung levels of MPO.

MATERIALS AND METHODS

Bilateral LC injury

All animal experiments followed protocols approved by the Institutional Animal Care and Use Committee at the State University of New York at Buffalo and complied with State, Federal, and National Institutes of Health regulations. Lung contusion injury was induced in halothane-anesthetized, adult, male, Long-Evans rats (250 – 300 g body weight; Harlan Sprague-Dawley, Indianapolis, Ind) by dropping a 300-g cylindrical weight 68 cm (impact energy, 2.0 J) onto a Lexon precordial shield placed on the chest of a supine rat (8,9). The path of the falling weight was controlled by a vertical cylindrical tube, and a precordial shield (with Teflon guides) directed the impact force bilaterally to the lungs while sparing the heart. Previous studies in this model have documented that rats have bilateral pulmonary contusion with minimal cardiac trauma by gross and histopathological analysis (8,9). In addition, the absence of rib fractures or pneumothoraces has been documented by chest radiography (8,9). The impact energy level of 2.0 J was chosen for the present study to ensure minimal mortality when LC was combined in a subset of studies with the added pulmonary challenge of gastric aspiration. Rats after LC were allowed to recover from anesthesia and were then maintained in room air before being evaluated at 24, 48, 72, or 96 h postcontusion. A total of 24 rats were studied for LC injury across these time points, and an additional 6 uninjured control rats were examined for baseline comparisons.

Gastric aspiration–induced lung injury (CASP)

Additional experiments investigated a clinically relevant combination injury of LC with and without concurrent gastric aspiration in 13 Long-Evans rats identical to those investigated for LC alone. Halothane-anesthetized rats given LC, as described previously, were allowed to recover in 100% oxygen until regular spontaneous breathing was obtained (a period of ~3 – 5 min). Animals were then immediately reanesthetized and instilled intratracheally with CASP (1.2 mL/kg of isotonic sodium chloride solution containing 40 mg/mL of small gastric food particles adjusted to a pH of 1.25 with hydrochloric acid). Gastric food particles were prepared from the stomach contents of healthy Long-Evans rats, washed in isotonic sodium chloride solution, and coarse-filtered through gauze (10). The distribution of particle diameters in equivalent CASP preparations has been reported by Knight et al. (14) to be bimodal, with peaks at approximately 4.5 and 13 μ m and a mean diameter of less than 10 μ m. The CASP injury vehicle was instilled through a 2" $\times$ 14-gauge angiocatheter inserted into the trachea transorally to approximately 0.5 cm above the carina, with anatomical placement verified by continuous end-tidal carbon dioxide tracing (RASCAL II Raman light scattering spectrophotometer; Ohmeda, Salt Lake City, Utah). Just before instillation of CASP, the chest wall was compressed by hand and rapidly released as the aspirate volume was injected into the endotracheal catheter with a 1-mL syringe followed by a 0.5-mL air bolus chaser to ensure complete delivery of the

aspirate and facilitate its rapid distal alveoli dissemination. Rats given LC + CASP or CASP were then maintained in room air until assessments of lung injury and surfactant at 24 h.

BAL procedure and cell counts

At the end of an experiment, animals were anesthetized with halothane, and a midline incision was made through the sternum. The lung vasculature was flushed by injecting 20 mL Hank balanced salt solution at 37°C into the beating right ventricle, and animals were exsanguinated by transecting the abdominal vena cava. Bronchoalveolar lavage was performed by injecting 5 × 10 mL of 37°C isotonic sodium chloride solution through a 14-gauge tracheal cannula inserted and secured in place with a suture. Recovered BAL fluid was centrifuged at 150g at 4°C for 10 min to pellet cells. The cell pellet was resuspended in 4 mL of phosphate-buffered isotonic sodium chloride solution + 0.1% sodium azide, and erythrocytes (red blood cells [RBCs]) and leukocytes were enumerated with a Multisizer 3 Coulter Counter (Beckman Coulter, Fullerton, Calif). Differential counts for PMNs were performed on pelleted cells after cytocentrifugation (Cytospin 3; Shandon Lipshaw, Pittsburgh, Pa) and staining with Diff-Quik (Baxter, Detroit, Mich).

Albumin, total protein, and total phospholipid concentrations in cell-free BAL

Albumin concentrations (in micrograms per milliliter) in cell-free BAL were measured by enzyme-linked immunosorbent assay with a polyclonal rabbit antimouse albumin antibody (generously provided by Dr. Daniel Remick, Boston University, Boston, Mass) and horseradish peroxidase-labeled goat antirabbit immunoglobulin G (BD Biosciences Pharmingen, San Diego, Calif) (11). Rat albumin (Sigma, St. Louis, Mo) was used as a standard. Total protein was measured in BAL by the method of Lowry et al. (18), modified by the addition of 15% sodium dodecyl sulfate to allow accurate quantitation in the presence of lipid. Total phospholipid in cell-free BAL was measured by the phosphorus assay of Ames (19).

Whole lung MPO activity

Whole lung MPO activity was studied as an added measure of neutrophil-associated pulmonary inflammation. After BAL, lungs were excised, and ice-cold isotonic sodium chloride solution with 1 × protease inhibitor the “cocktail” (500 μM AEBSF HCl, 150 nM aprotinin, 1 μM E-64, 0.5 mM EDTA disodium, 1 μM leupeptin hemisulfate final concentrations; Calbiochem, LA Jolla, Calif) was added to a total weight of 10 g (tissue + saline). The lungs were homogenized on ice with a Polytron TP-2000 tissue homogenizer (Brinkman Instruments, Westbury, NY). The tissue homogenate was centrifuged at 40,000 g for 10 min at 4°C, and MPO was extracted from the pellet by resuspension in 5 mL phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethylammonium bromide and 5 mM EDTA, followed by three freeze-thaw-sonication cycles (1 min, 50% duty cycle Branson Sonifier with microtip probe; Branson Ultrasonics, Danbury, Conn). The suspension was centrifuged again at 40,000g for 10 min, and the supernatant was combined with the supernatant from a second MPO extraction of the pellet. Final MPO activity was measured by combining 10 μL of extracted sample with 300 μL of assay buffer (pH 6.0, containing 50 mM KH₂PO₄, 176 mM H₂O₂, and 52.5 mM *o*-dianisidine dihydrochloride) in a 96-well microplate (Sarstedt, Newton, NC). Absorbance was recorded at 460 nm for 90 s at 2-s intervals using a SPECTRAmax 190 plate reader (Molecular Devices, Sunnyvale, Calif). Myeloperoxidase activity was expressed in arbitrary units (defined as the absorbance change per minute over linear portion of the curve) and was normalized to the total volume of extracted sample recovered from the whole lung (10).

Content and composition of centrifuged large surfactant aggregates

Large surfactant aggregates were sedimented by centrifugation of cell-free BAL at 12,000 g for 30 min. Large aggregates obtained under similar centrifugation conditions have been used

in multiple previous studies as a reflection of biophysically active endogenous lung surfactant ([20], for review). The content of large surfactant aggregates as a percentage of total phospholipid in cell-free BAL from rats with LC or LC + CASP was determined by phosphate assay (19), and total protein in aggregates was measured by the sodium dodecyl sulfate–modified assay of Lowry et al. (18) as in whole BAL protein measurements earlier. The hydrophobic protein content of large aggregates was measured after extraction into chloroform (21). Because most biological proteins are not chloroform soluble, most chloroform-extractable BAL protein is composed of surfactant proteins B and C (20,22,23). In addition to protein measurements in large aggregates, phospholipid classes, including lyso-PC, were defined by thin layer chromatography using a solvent system of chloroform-methanol-2-propanol-triethylamine-water (30:9:25:25:7 by volume) (24).

Pulsating bubble measurements of large aggregate surface activity

The surface activity of resuspended large surfactant aggregates was assessed during cycling at a physiological rate of 20 cycles per minute at $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ on a pulsating bubble surfactometer (General Transco, Largo, Fla) (25). A small air bubble, communicating with ambient air, was formed in a 40- μL aliquot of BAL large surfactant aggregate in a plastic sample chamber mounted on the pulsator unit of the surfactometer. The bubble was oscillated between maximum and minimum radii of 0.55 and 0.4 mm (50% area compression for a truncated sphere), whereas the pressure drop across the air-water interface was measured with a precision pressure transducer. Surface tension at minimum bubble radius (minimum surface tension) was calculated as a function of time of pulsation from the measured pressure drop at end-compression and the Laplace equation for a spherical interface (25,26). Bronchoalveolar lavage large surfactant aggregate samples were examined at a uniform phospholipid concentration of 1 mg/mL in 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 2 mM CaCl_2 , and 150 mM NaCl, pH 7.0.

Exogenous surfactant replacement

To document the functional importance of surfactant abnormalities in LC injury, pilot studies of exogenous surfactant replacement were done at 24 h postcontusion with the clinical surfactant drug Infasurf (calf lung surfactant extract; ONY, Inc., Amherst, NY; dose, 100 mg/kg body weight) (20,27,28). The 24 h post-LC time point of surfactant therapy was chosen for pilot studies because untreated rats exhibited the most severe surfactant dysfunction at this time. Quasistatic pressure-volume (P-V) inflation/deflation mechanics and BAL albumin were assessed at 4 h postsurfactant delivery compared with untreated LC rats and uninjured controls. A 14-gauge steel tracheal cannula was inserted and secured by a suture in halothane-anesthetized Long-Evans rats through a 2-cm ventral midline neck incision, and animals were exsanguinated by transection of the abdominal inferior vena cava. Air was injected into the lungs at a rate of 25 mL/min by a syringe pump connected to the tracheal cannula. Inflation pressure was monitored continuously by an inline transducer connected to an Apple PowerBook G4 (Apple Computer, Cupertino, Calif) equipped with a National Instruments data acquisition board (Austin, Tex) and custom-written Lab VIEW 6.0 software (National Instruments). When pressure reached 40 cm H_2O , the syringe pump was reversed, and deflation pressures were monitored. Volumes were calculated based on the rate of injection or withdrawal and were normalized to kilogram body weight. After P-V measurements, lungs were immediately lavaged, and albumin concentrations were measured using previously described methods.

Statistical analyses

All statistical analyses were performed using the statistical software JMP IN v.5.1 (SAS Institute Inc., Cary, NC). Initially, data within each group for each parameter were tested for

normal distribution using the Shapiro-Wilk *W* test. Because several parameters exhibited nonnormal distribution and the modest sample size, results were analyzed using the Kruskal-Wallis rank-sum test. If the null hypothesis (i.e., all group means being the same) was rejected ($P < 0.05$), then pair-wise comparisons between each group were made using an analog of the Bonferroni pair-wise comparison based on observation ranks.

The two-sided family level of significance for all comparisons was set at $\alpha = 0.05$. Adjusting for multiple comparisons, individual comparisons assessing LC at 5 time points (i.e., 0, 24, 48, 72, and 96 h, yielding 10 comparisons) were considered significant if $P < 0.0025$. Experiments that involved comparing the four injury modalities (i.e., uninjured, LC, CASP, and LC + CASP, yielding six comparisons) were considered significant if $P < 0.0042$. Spearman ρ correlations were also calculated to examine correlative relationships between specific parameters of surfactant dysfunction and the severity of lung injury. Descriptive statistics for each outcome were expressed as mean $\pm$ SEM, as well as the median. Box plots display the 25th and 75th quartiles of the data, with the bars indicating the range. The horizontal bar within the quartile box denotes the median of the data, and the filled square symbol denotes the mean.

RESULTS

Lung injury severity in rats with LC injury at different times

Rats with bilateral LC injury had substantial inflammatory injury that was most severe at 24 h postcontusion then improved with time (Table 1). At 24 h postcontusion, there was significant injury at the level of the alveolar-capillary membrane as reflected in BAL concentrations of total protein and albumin that were 19- and 7-fold higher, respectively, than those in uninjured control rats ($P < 0.0001$; Table 1). The protein to phospholipid ratio in BAL was increased by more than an order of magnitude in rats with LC injury at 24 h compared with controls. In addition, numbers of RBCs in BAL were greatly elevated for rats with LC injury at 24 h ($1.5 \pm 0.4 \times 10^8$ cells vs. $4.9 \pm 1.7 \times 10^5$ cells in BAL from uninjured controls; $P < 0.0001$). Whole lung MPO activity was also increased in rats at 24 h postcontusion, consistent with increased neutrophil-associated pulmonary inflammation compared with uninjured controls. Lung injury was still apparent in rats at 48 and 72 h postcontusion, although with a reduced severity compared with 24 h (Table 1). At 96 h postcontusion, some lung injury parameters were slightly elevated over control values (BAL total protein and RBCs), but many were indistinguishable from controls (BAL albumin and protein-phospholipid ratio, and whole lung MPO). We have previously reported detailed data on arterial oxygenation, P-V mechanics, and BAL inflammatory mediator concentrations in an identical rat LC model (8,9), and these measurements were not repeated here. These previous results showed that rats with LC had $\text{PaO}_2/\text{FIO}_2$ ratios of 300 to 435 torr at 24 to 72 h postcontusion (hypoxemia in this model is most severe in the first 6 h after contusion) (8,9). However, the pulmonary inflammatory response peaked in rats with LC injury at 24 to 48 h (8,9), and these postcontusion times were thus examined in the current article to assess surfactant dysfunction related to pulmonary inflammation and to permeability injury.

Abnormalities in large aggregate content and composition in LC injury

Rats with LC injury had significant surfactant dysfunction in large aggregate content, composition, and surface activity at 24, 48, and 72 h postcontusion (Fig. 1 and Fig. 2, Table 2 and Table 3). Consistent with the lung injury severity findings previously discussed, surfactant dysfunction was most severe at 24 h postcontusion and improved with time thereafter. Although levels of total BAL phospholipid were not significantly different in rats at 24 h after LC compared with uninjured controls, the percentage of lavaged phospholipid that was in the form of large surfactant aggregates was decreased significantly at this time. At 24 h post-LC, the large surfactant aggregate fraction of BAL made up only $22.6\% \pm 2.5\%$ of total BAL

phospholipid compared with $48.7\% \pm 1.4\%$ for uninjured controls ($P < 0.0001$; Fig. 1). The percentage of large surfactant aggregates in BAL at 48 and 72 h after LC was also reduced compared with control ($33.8\% \pm 1.5\%$ at 48 h and $37.9\% \pm 2.1\%$ for 72 h; $P < 0.0001$; Fig. 1). At 96 h postcontusion, the percentage of large aggregates in cell-free BAL reached control levels (Fig. 1).

Large surfactant aggregates obtained by centrifugation of cell-free BAL from rats with LC injury had several abnormalities in protein and phospholipid composition (Table 2 and Table 3). Large aggregates from rats with LC had substantially greater levels of total protein (weight % relative to total large aggregate phospholipid; $P < 0.0001$) at 24, 48, and 72 h postcontusion compared with uninjured controls (Table 2). This effect was most pronounced in large aggregates from rats at 24 h after LC, which had a total large aggregate protein content that was 5-fold higher than in control large aggregates from uninjured rats (Table 2). The protein content of large aggregates from rats with LC injury was also increased at 48 and 72 h postcontusion but to a lesser degree than at 24 h (Table 2). Most of the observed increases in large aggregate total protein content were due to increased amounts of hydrophilic (chloroform-insoluble) protein because levels of hydrophobic (chloroform-soluble) protein in centrifuged aggregates remained at a low level of less than 2% by weight, and no statistically significant differences were detected between injury groups (Table 2). In addition to having altered protein content, large aggregates from rats had significant abnormalities in phospholipid composition (Table 3). In particular, large aggregates from animals with LC exhibited decreased levels of PC, increased levels of lyso-PC, and increased levels of cell-associated phospholipids (sphingomyelin + phosphatidylethanolamine) compared with large aggregates from uninjured controls. These changes were again greatest for rats at 24 h post-LC injury, with subsequent improvement and a return to levels measured in uninjured control rats by 96 h (Table 3).

Abnormalities in large aggregate surface activity in LC injury

In addition to having abnormal phospholipid and total protein contents, large aggregates were also significantly impaired in surface activity after LC injury (Fig. 2). Centrifuged large aggregates were resuspended in buffered saline at a uniform phospholipid concentration of 1 mg/mL and studied for overall dynamic surface tension-lowering ability on a pulsating bubble surfactometer. Large aggregates obtained at 24 h after LC had a greatly increased time course of surface tension lowering and reached minimum surface tensions of only 15.6 ± 1.1 mN/m after prolonged cycling (20 min) on the bubble surfactometer compared with minimum surface tensions of 0.7 ± 0.3 mN/m for uninjured controls (Fig. 2). Minimum surface tensions after 20 min of bubble pulsation were also elevated for resuspended large aggregates obtained at 48 and 72 h postcontusion (9.1 ± 1.9 and 5.5 ± 1.7 mN/m, respectively) (Fig. 2). Aggregates obtained at 96 h after LC had minimum surface tension values after 20 min of pulsation that were equivalent to those found of uninjured controls (1.2 ± 0.3 mN/m).

Surfactant replacement in rats with LC injury

To document the functional importance of surfactant abnormalities in rats with LC, pilot experiments were done on exogenous surfactant replacement therapy at 24 h postcontusion when deficits in endogenous surfactant were maximal. Rats were treated with Infasurf (100 mg/kg body weight) at this time and were then assessed for quasistatic closed chest P-V mechanics and albumin in BAL at 4 h postsurfactant. Rats given Infasurf had significantly improved P-V inflation/deflation curves compared with rats not receiving surfactant (Fig. 5A). Surfactant treatment at 24 h post-LC improved deflation P-V mechanics essentially to the level of uninjured control rats and also improved inflation P-V mechanics at transpulmonary pressures of 15 to 40 cm H₂O. Surfactant-treated LC rats had a total lung volume at 40 cm H₂O pressure equal to uninjured rats and significantly greater than LC rats not treated with surfactant (37.7 ± 0.6 mL/kg for surfactant-treated rats vs. 33.9 ± 0.5 mL/kg for untreated

contused rats; $P < 0.001$; Fig. 5B). The mean concentration of albumin in BAL was lower in surfactant-treated ($410 \pm 113 \mu\text{g/mL}$) versus untreated ($645 \pm 112 \mu\text{g/mL}$) rats with LC injury, although these albumin differences did not reach full statistical significance ($P = 0.07$).

Lung injury severity and surfactant dysfunction in rats with LC + CASP compared with LC and CASP alone

Additional experiments investigated the aspiration of gastric acid and food particles (CASP) as a clinically relevant insult that can occur in conjunction with blunt trauma-induced LC. Experimental assessments of lung injury and surfactant dysfunction in LC + CASP were done at the 24-h postinjury time point where abnormalities were most prominent in rats given LC alone. Rats with LC + CASP had the most severe lung injury, with an increased BAL protein/phospholipid ratio compared with rats given LC or CASP alone (Table 4). Total protein, albumin, and RBCs in BAL were also significantly higher in rats given LC + CASP compared with CASP alone, but not LC alone at the 24-h postinjury time point. Large aggregate content as a percent of total BAL phospholipid was decreased to a similar extent in all three injury groups (Fig. 3), and all groups also exhibited abnormalities in large aggregate composition and surface activity (Fig. 4 and Table 5). However, several aggregate-related variables were most abnormal in rats with the combination injury of LC + CASP compared with LC or CASP alone. In particular, large aggregate lyso-PC and total protein content were highest, and large aggregate PC levels were lowest, for rats given LC + CASP (Table 5). Minimum surface tension values for large aggregates after 20 min of pulsation in the bubble surfactometer were significantly higher for rats with LC + CASP compared with LC alone ($20.9 \pm 0.6 \text{ mN/m}$ for LC + CASP vs. $15.6 \pm 1.1 \text{ mN/m}$ for LC; $P = 0.0001$; Fig. 4). In addition, minimum surface tensions for large aggregates from rats given LC + CASP were substantially higher than those for CASP at bubble pulsation times of 5 min or less (Fig. 4).

Correlation analyses for variables assessing surfactant dysfunction and lung injury severity

Pair-wise correlation analyses were used to assess the degree of interaction between surfactant dysfunction and the severity of lung injury in all animal groups and time points studied. Although these correlations could not directly assess causality, they did document a strong association between specific variables of surfactant dysfunction and the severity of permeability injury (BAL total protein, albumin, protein/phospholipid ratio), hemorrhagic injury (BAL RBCs), and pulmonary inflammation (BAL levels of PMNs and whole lung MPO activity). There were strong correlations between the extent of surfactant dysfunction based on large aggregate content and minimum surface tension at 20 min of bubble pulsation and all of these variables ($P < 0.0001$ for all correlations; Table 6). In addition, the percentage of large aggregate total protein and lyso-PC correlated similarly with these injury parameters ($P < 0.0001$ for all correlations; Table 7).

DISCUSSION

This study demonstrates for the first time that the time-dependent pathophysiology of pulmonary contusion includes functionally important reductions in the content and surface activity of large surfactant aggregates (Fig. 1 and Fig. 2). Large surfactant aggregates from rats with LC injury also had altered composition, with increased levels of protein and lysoPC and decreased levels of PC (Table 2 and Table 3). Surfactant dysfunction in LC injury was most severe at 24 h postcontusion but was also apparent at 48 and 72 h postcontusion, consistent with the evolution of lung injury severity variables (Table 1). Pilot exogenous surfactant replacement studies at 24 h post-LC showed significantly improved inflation/deflation P-V mechanics in rats treated with Infasurf compared with untreated rats with LC (Fig. 5). In addition, there was a trend toward reduced BAL albumin levels in rats given Infasurf ($P = 0.07$). Although these pilot surfactant replacement results need to be extended in more detailed

future studies, they document the functional importance of surfactant abnormalities in LC injury.

Reductions in the content, composition, and surface activity of large surfactant aggregates were also documented at 24 h postinjury in rats given a combination of LC and gastric aspiration (LC + CASP; Fig. 3 and Fig. 4 and Table 5). Rats with LC + CASP had increased protein-phospholipid ratios in BAL compared with rats with LC or CASP alone (Table 4), and increased surfactant dysfunction in reduced large aggregate surface activity, increased large aggregate protein and lyso-PC, and decreased aggregate PC compared with rats given LC or CASP alone (Fig. 4 and Table 5). Pair-wise correlation analyses across all animal groups (LC, LC + CASP, CASP, and control) and injury times studied showed that indices of surfactant dysfunction (large aggregate content in BAL, minimum surface tension at 20 min of bubble pulsation, and large aggregate protein and lyso-PC contents) correlated strongly with lung injury severity based on BAL albumin, total protein, protein-phospholipid ratio, numbers of RBCs, and PMNs (Table 6 and Table 7). There was also a significant correlation between whole lung MPO activity as a measure of neutrophil-associated pulmonary inflammation and indices of surfactant dysfunction (Table 6 and Table 7).

Previous studies have not investigated surfactant dysfunction in animals with closed chest LC injury despite the clinical relevance of this condition. In a small clinical study in 1999, Aufmkolk et al. (29) reported analyses of BAL fluid obtained during 7 days from 14 trauma patients with unilateral pulmonary contusion that showed a significant increase in total BAL phospholipid plus a slight increase in sphingomyelin and a decrease in phosphatidylglycerol in BAL from the contused lung compared with the contralateral (unaffected) lung. However, large surfactant aggregate content, composition, and activity were not assessed. Our results for phospholipid classes (Table 3) and surface activity (Fig. 2) are not comparable to those of Aufmkolk et al. (29) because we assessed centrifuged large surfactant aggregates as opposed to whole lavage. The large aggregate fraction of BAL studied here is particularly relevant for functional lung surfactant because large aggregates are known to have the greatest surface activity and the highest apoprotein content in endogenous surfactant (20,30–33). Decreases in the content and surface activity of large surfactant aggregates from rats with LC (Fig. 1 and Fig. 2) and LC + CASP (Fig. 3 and Fig. 4) in the current study are consistent with similar findings reported in animal models of inflammatory ALI/ARDS induced by gastric aspiration (11), *Escherichia coli* pneumonia (34), *Pneumocystis carinii* pneumonia (35), thoracic radiation/bone marrow transplant (36), and *N*-nitroso-*N*-methylurethane injury (37). Reductions in large aggregate content and/or activity have also been reported in BAL from patients with ALI/ARDS (38–40). The present study extends this previous work to show severe surfactant dysfunction in rats with LC and with LC + CASP, with the latter being particularly severe in aggregate compositional deficits (Table 5).

There are multiple mechanistic pathways by which lung surfactant abnormalities can be generated during LC and LC + CASP. One mechanism that almost certainly contributes to the surface activity decreases observed here involves biophysical inactivation of surfactant by blood-derived proteins such as albumin (41–44). Another blood protein of likely relevance for our findings is hemoglobin, which has also been shown to inhibit surfactant activity *in vitro* (41,45). Erythrocytes were present in substantial amounts in the contused lungs, and their numbers in BAL correlated significantly with deficits in large aggregate surface activity and composition (Table 6 and Table 7). Albumin and total protein concentrations in BAL also correlated strongly with decreased large aggregate surface activity and compositional abnormalities (Table 6 and Table 7). In isolated LC injury, albumin and total protein were elevated to the greatest extent at 24 h postcontusion (Table 1), and rats with LC had the greatest reductions in large aggregate surface activity at this time (Fig. 2). Albumin, hemoglobin, and related blood proteins impair lung surfactant activity in part by competitive adsorption that

reduces the concentration of active surfactant components in the surface film (20,43,46). In addition, blood-derived proteins can also physically incorporate into surfactant aggregates to impair their activity. The large increases found here in total protein in large aggregates from rats with LC and LC + CASP (Table 2 and Table 5) are consistent with blood-derived proteins being incorporated into or becoming associated with surfactant aggregates in the alveolar hypophase. Protein increases in large aggregates did not involve hydrophobic (chloroform-extracted) protein, which was unchanged in injured animals (Table 2 and Table 5). It is possible that some of the increase in large aggregate protein included hydrophilic surfactant apoproteins expressed during inflammatory injury. However, the strong statistical correlation found between total protein in aggregates and total protein/albumin in BAL (Table 7) indicates that most of the increased protein in the aggregates was injury-derived nonsurfactant protein present as a result of permeability injury.

A second mechanism contributing to reductions in large aggregate surface activity involved changes in phospholipid composition, particularly decreased amounts of PC and increased amounts of lyso-PC (Table 3 and Table 5). Phosphatidylcholine compounds make up approximately 80% of total surfactant phospholipids in normal animals (20,47,48) and include crucial disaturated molecular species such as dipalmitoyl PC, which generate very low surface tensions in compressed interfacial films (20,47). Decreases in large aggregate PC to 65.6% to 73.5% in rats with LC + CASP, LC, and CASP at 24 h postinjury (Table 5) thus have functional relevance. The increased level of lyso-PC found in large aggregates from injured animals (Table 3 and Table 5) is consistent with the degradation of surfactant PC to lyso-PC by phospholipases in the pulmonary inflammatory response. Lysophosphatidylcholine is a known biophysical inhibitor of surfactant activity that penetrates directly into the interfacial film to impair surface tension lowering during dynamic compression (20,43,44).

Lysophosphatidylcholine has also been shown to generate additive surfactant inhibition when present together with albumin (44). The increased levels of lyso-PC in large aggregates in this study suggest that quantitative measurements of pulmonary phospholipase activity would be of interest in future investigations. Moreover, in addition to surfactant dysfunction from biophysical inhibitors and chemical degradation, other inflammation-induced mechanisms are also likely to be relevant for our results. We have previously shown that multiple inflammatory mediators are elaborated during LC injury (8,9) and CASP aspiration (10,12,15), and inflammation-associated surfactant dysfunction has also been reported in other animal models of ALI/ARDS (35,36,49). Correlations were found here between surfactant dysfunction and the inflammation-related variables of whole lung MPO activity and BAL numbers of PMNs (Table 7).

For physiological relevance, the presence of surfactant dysfunction in rats with LC and LC + CASP agrees with our previous studies documenting that pulmonary volumes and compliance are significantly reduced during the course of both LC injury (9) and CASP injury (11). Our pilot exogenous surfactant replacement results showing that compliance in animals with LC injury can be markedly improved by the administration of Infasurf at 24 h postcontusion document the physiological importance of surfactant abnormalities in this condition as noted earlier. Using an open chest model for LC injury in pigs, Strohmaier et al. (50) have reported significant improvements in lung function after bilateral lavage with the porcine surfactant Curosurf. The bovine surfactant extract Infasurf used in our experiments has recently been shown to improve pulmonary function and survival in pediatric patients up to age 21 years with ALI/ARDS, with the greatest benefits found in patients with direct pulmonary injury as opposed to indirect, nonpulmonary causes (28). More detailed future studies of surfactant supplementation therapy in rats with LC or LC + CASP will be important in determining the use of this approach in mitigating these clinically important direct pulmonary causes of ALI/ARDS.

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FIG. 1. Content of large surfactant aggregates in cell-free BAL fluid from rats at various times after LC injury

The data represent the fraction of the total BAL phospholipid that sedimented as large surfactant aggregate obtained by centrifugation at 12,000 *g* for 30 min. Box plots display the 25th and 75th quartiles of the data, with the bars indicating the range. The horizontal bar within the quartile box denotes the median of the data, and the filled square symbol denotes the mean (*n* = 6 rats in each group). Large aggregates were depleted in LC injury at all time points except 96 h compared with controls, with the greatest decrease in large aggregate content at 24 h. Significant differences from a specific group are indicated by *uninjured; #LC 24 h; †LC 48 h; and §LC 72 h. Adjusting for multiple comparisons, differences were considered significant if *P* < 0.0025 to maintain a family-wise α error less than 0.05.

FIG. 2. Surface activity of resuspended large surfactant aggregates from rats with LC injury at various durations after LC injury

Centrifuged large surfactant aggregates obtained at 24 to 96 h post-LC were resuspended at a uniform phospholipid concentration of 1 mg/mL in 10 mM HEPES, 2 mM CaCl_2 , and 150 mM NaCl, pH 7.0, and examined for surface activity on a pulsating bubble surfactometer (37°C; 20 cycles per minute; 50% area compression). A, Surface tension at minimum bubble radius (minimum surface tension) as a function of time of pulsation. B, Minimum surface tension after 20 min of pulsation. Box plots display the 25th and 75th quartiles of the data, with the bars indicating the range. The horizontal bar within the quartile box denotes the median of the data, and the filled square symbol denotes the mean ($n = 6$ rats in each group). The minimum

surface tension at 20 min of pulsation was elevated at 24 to 72 h postcontusion compared with uninjured controls, with the greatest elevation occurring at 24 h post-LC. Significant differences from a specific group are indicated by *uninjured; #LC 24 h; †LC 48 h; and §LC 72 h. Adjusting for multiple comparisons, differences were considered significant if $P < 0.0025$ to maintain a family-wise α error less than 0.05.

FIG. 3. Content of large surfactant aggregates in cell-free BAL fluid from rats at 24 h after different pulmonary injuries: LC, CASP, or LC + CASP

The data represent the fraction of the total BAL phospholipid that sedimented as large surfactant aggregate obtained by centrifugation at 12,000g for 30 min. Box plots display the 25th and 75th quartiles of the data, with the bars indicating the range. The horizontal bar within the quartile box denotes the median of the data, and the filled square symbol denotes the mean ($n = 5 - 7$ rats in each group). Large aggregates were depleted in all lung injuries at 24 h compared with uninjured controls. Significant differences from a specific group are indicated by *uninjured and #LC 24 h. Adjusting for multiple comparisons, differences were considered significant if $P < 0.0025$ to maintain a family-wise α error less than 0.05.

FIG. 4. Surface activity of resuspended large surfactant aggregates from rats with LC injury at 24 h after different pulmonary injuries: LC, CASP, or LC + CASP

Centrifuged large surfactant aggregates from each injury group at 24 h postinsult were resuspended at a uniform phospholipid concentration of 1 mg/mL in 10 mM HEPES, 2 mM CaCl_2 , 150 mM NaCl, pH 7.0, and examined for surface activity on a pulsating bubble surfactometer (37°C, 20 cycles per minute, 50% area compression). A, Minimum surface tension as a function of duration of pulsation. B, Minimum surface tension after 20 min of pulsation. Box plots display the 25th and 75th quartiles of the data, with the bars indicating the range. The horizontal bar within the quartile box denotes the median of the data, and the filled square symbol denotes the mean ($n = 5 - 7$ rats in each group). The minimum surface

tension after 20 min of pulsation was elevated in all lung injuries tested compared with uninjured controls, with injuries containing CASP resulting in a greater elevation than LC alone. Significant differences from a specific group are indicated by *uninjured and #LC 24 h. Adjusting for multiple comparisons, differences were considered significant if $P < 0.0025$ to maintain a family-wise α error less than 0.05.

FIG. 5. Inflation/deflation P-V mechanics in rats treated with exogenous surfactant at 24 h postcontusion injury in pilot studies

Surfactant-treated rats received Infasurf (100 mg/kg body weight) by intratracheal instillation at 24 h post-LC, and quasistatic P-V curves were measured at 4 h postsurfactant. Rat groups shown are LC injury (surfactant treated), LC injury (no treatment), and uninjured controls. A, Complete inflation-deflation P-V curves. B, Total lung volume at 40 cm H₂O pressure (mean $\pm$ SEM; n = 5 animals per group). #*P* < 0.001 compared with rats with LC that received therapy with Infasurf or to uninjured controls.

TABLE 1
Biochemical analysis of whole cell-free BAL, enumeration of RBC extravasation into the lung air space (BAL RBCs), and whole lung MPO activity from rats at various times after LC injury

Injury group	BAL total protein, $\mu\text{g/mL}$	BAL albumin, $\mu\text{g/mL}$	BAL total PL, $\mu\text{g/mL}$	BAL total protein-PL ratio, %	BAL RBCs	Whole lung MPO, units per lung
Uninjured	38 $\pm$ 2 (38)	4.9 $\pm$ 0.4 (5.1)	34.9 $\pm$ 1.9 (33.6)	110 $\pm$ 7 (117)	4.9 $\pm$ 1.7 $\times 10^5$ (3.9 $\times 10^5$)	0.15 $\pm$ 0.02 (0.14)
LC 24 h	726 $\pm$ 154 (612) [*]	36.2 $\pm$ 6.5 (31.5) [*]	54.0 $\pm$ 8.6 (49.0)	1,280 $\pm$ 90 (1,299) [*]	1.5 $\pm$ 0.4 $\times 10^8$ (1.2 $\times 10^8$) [*]	1.16 $\pm$ 0.31 (0.92) [*]
LC 48 h	184 $\pm$ 40 (210) ^{*,†}	20.4 $\pm$ 3.6 (22.3) [*]	41.7 $\pm$ 3.4 (38.8)	435 $\pm$ 90 (486) ^{*,†}	2.4 $\pm$ 1.1 $\times 10^7$ (1.4 $\times 10^7$) ^{*,†}	0.51 $\pm$ 0.11 (0.51) ^{*,†}
LC 72 h	92 $\pm$ 8 (93) ^{*,†}	17.5 $\pm$ 3.6 (17.0) ^{*,†}	36.1 $\pm$ 7.0 (38.2)	336 $\pm$ 67 (267) ^{*,†}	1.3 $\pm$ 0.5 $\times 10^7$ (9.0 $\times 10^6$) ^{*,†}	0.34 $\pm$ 0.06 (0.31) ^{*,†}
LC 96 h	51 $\pm$ 8 (52) ^{*,†,‡,§}	7.3 $\pm$ 1.0 (6.9) ^{†,‡,§}	38.7 $\pm$ 2.4 (39.8)	130 $\pm$ 16 (125) ^{†,‡,§}	3.6 $\pm$ 1.1 $\times 10^6$ (2.8 $\times 10^6$) ^{*,†,‡,§}	0.16 $\pm$ 0.03 (0.16) ^{†,‡,§}

Data are mean $\pm$ SEM (median), with n = 6 rats in each group. Significant differences from a specific group are indicated by

^{*} uninjured

[†] LC 24 h

[‡] LC 48 h

[§] LC 72 h. Adjusting for multiple comparisons, differences were considered significant if $P < 0.0025$ to maintain a family-wise α error less than 0.05.

PL indicates phospholipid

TABLE 2

Total protein and hydrophobic protein in large surfactant aggregates centrifuged from cell-free BAL from rats at various times after LC injury

Injury group	Large aggregate total protein, wt % relative to phospholipid	Large aggregate hydrophobic protein, wt % relative to phospholipid
Uninjured	11.6 ± 0.9 (12.3)	1.63 ± 0.05 (1.63)
LC 24 h	58.1 ± 3.6 (60.1) *	1.96 ± 0.02 (1.96)
LC 48 h	39.2 ± 5.5 (43.8) *, [†]	1.84 ± 0.07 (1.84)
LC 72 h	31.9 ± 5.6 (28.7) *, [†]	1.72 ± 0.1 (1.72)
LC 96 h	14.3 ± 1.0 (14.1) [†] , [‡] , [§]	1.57 ± 0.02 (1.57)

Large surfactant aggregates were pelleted from cell-free BAL by centrifugation at 12,000g for 30 min, and protein was measured with (hydrophobic protein) and without (total protein) extraction into chloroform and presented as the weight percent of total BAL phospholipid. Data are mean ± SEM (median), with n = 6 rats in each group. Significant differences from a specific group are indicated by

* uninjured

[†] LC 24 h

[‡] LC 48 h

[§] LC 72 h. Adjusting for multiple comparisons, differences were considered significant if $P < 0.0025$ to maintain a family-wise α error less than 0.05. Hydrophobic protein values did not differ significantly among any of the groups.

Phospholipid class compositions in large surfactant aggregates centrifuged from cell-free BAL from rats at various times after LC injury

TABLE 3

Injury group	Phospholipid class composition in large surfactant aggregates (%)						
	PC	Lyso-PC	SPH	PI + PS	PE	PG	Residue
Uninjured	84.3 ± 0.5 (84.2)	1.2 ± 0.2 (1.1)	2.3 ± 0.5 (2.5)	2.7 ± 0.1 (2.6)	1.6 ± 0.2 (1.8)	7.1 ± 0.1 (7.1)	0.7 ± 0.1 (0.8)
LC 24 h	73.5 ± 0.8 (73.4)	6.0 ± 0.1 (6.0)*	5.0 ± 0.3 (4.9)*	5.4 ± 0.5 (5.8)*	4.1 ± 0.4 (4.2)*	4.9 ± 0.1 (4.8)*	1.1 ± 0.2 (1.0)
LC 48 h	76.7 ± 1.2 (75.8)	4.1 ± 0.3 (4.2)*	4.7 ± 0.4 (4.7)*	4.2 ± 0.3 (4.3)* [†]	4.0 ± 0.4 (4.4)*	5.6 ± 0.2 (5.5)*	0.7 ± 0.2 (0.7)
LC 72 h	78.8 ± 0.7 (78.8)	2.1 ± 0.1 (2.2)* [†]	4.3 ± 0.2 (4.3)* [†]	4.3 ± 0.2 (4.3)*	3.6 ± 0.3 (3.5)*	6.1 ± 0.2 (6.3)* [†]	0.8 ± 0.1 (0.8)
LC 96 h	81.3 ± 0.6 (81.3)	1.5 ± 0.1 (1.6) ^{†,‡}	3.2 ± 0.2 (3.1) ^{†,‡,§}	3.4 ± 0.2 (3.4) ^{†,‡,§}	2.8 ± 0.4 (2.8)* ^{†,‡}	6.9 ± 0.1 (6.9) ^{†,‡,§}	0.9 ± 0.1 (1.0)

Large surfactant aggregates were pelleted from cell-free BAL by centrifugation at 12,000g for 30 min, and phospholipid class compositions were determined by thin layer chromatography. Phospholipid class expressed as weight percent relative to large aggregate total phospholipid content. Data are mean ± SEM (median), with n = 4 rats in each group. Significant differences from a specific group are indicated by

* uninjured

[†] LC 24 h

[‡] LC 48 h

[§] LC 72 h. Adjusting for multiple comparisons, differences were considered significant if $P < 0.0025$ to maintain a family-wise α error less than 0.05. PE indicates phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; SPH, sphingomyelin.

TABLE 4
Biochemical analysis of whole cell-free BAL, enumeration of RBC extravasation into the lung air space (BAL RBCs), and whole lung MPO activity from rats at 24 h after different pulmonary injuries: LC, CASP, or LC + CASP

Injury group	BAL total protein, $\mu\text{g/mL}$	BAL albumin, $\mu\text{g/mL}$	BAL total PL, $\mu\text{g/mL}$	BAL total protein to PL ratio, %	BAL RBCs	Whole lung MPO, units per lung
Uninjured	38 ± 2 (38)	4.9 ± 0.4 (5.1)	34.9 ± 1.9 (33.6)	110 ± 7 (117)	$4.8 \pm 1.7 \times 10^5$ (3.9×10^5)	0.15 ± 0.02 (0.14)
LC	726 ± 154 (612) [*]	36.2 ± 6.5 (31.5) [*]	54.0 ± 8.6 (49.0)	$1,280 \pm 120$ (1300) [*]	$1.5 \pm 0.4 \times 10^8$ (1.2×10^8) [*]	1.16 ± 0.31 (0.92) [*]
CASP	351 ± 90 (302) [*]	32.6 ± 5.1 (28.6) [*]	41.0 ± 6.3 (38.9)	906 ± 180 (911) [*]	$2.7 \pm 0.7 \times 10^7$ (2.7×10^7) ^{*,†}	1.99 ± 0.17 (1.91) [*]
LC + CASP	814 ± 100 (797) ^{*,†}	52.8 ± 5.6 (51.4) ^{*,†}	45.0 ± 4.1 (48.3)	$1,790 \pm 120$ (1,850) ^{*,†,‡}	$3.9 \pm 1.1 \times 10^8$ (2.5×10^8) ^{*,†}	1.57 ± 0.32 (1.73) [*]

Data are mean $\pm$ SEM (median), with n = 6 to 7 rats in each group. Significant differences from a specific group are indicated by
^{*} uninjured
[†] LC, or
[‡] CASP. Adjusting for multiple comparisons, differences were considered significant if $P < 0.0042$ to maintain a family-wise α error less than 0.05. PL indicates phospholipid.

TABLE 5

Changes in total protein, hydrophobic protein, and phospholipid class composition in large surfactant aggregates from centrifuged cell-free BAL from rats at 24 h after different pulmonary injuries: LC, CASP, or LC + CASP

Injury group	Large aggregate total protein, wt % relative to phospholipid	Large aggregate hydrophobic protein, wt % relative to phospholipid	Large aggregate PC content, % total phospholipid	Large aggregate lyso-PC content, % total phospholipid
Uninjured	11.6 ± 0.9 (12.3)	1.63 ± 0.05 (1.63)	84.3 ± 0.5 (84.2)	1.2 ± 0.2 (1.1)
LC	58.1 ± 3.6 (60.1) *	1.96 ± 0.02 (1.96)	73.5 ± 0.8 (73.4)	6.0 ± 0.1 (6.0)
CASP	43.7 ± 3.7 (47.1) *	1.68 ± 0.14 (1.68)	68.6 ± 0.5 (68.7) *	9.9 ± 0.5 (9.6) *
LC + CASP	96.2 ± 7.5 (89.6) * ^{†‡}	1.94 ± 0.02 (1.94)	65.6 ± 0.9 (65.5) * [†]	13.8 ± 0.7 (13.3) * [†]

Large surfactant aggregates were pelleted from cell-free BAL by centrifugation at 12,000g for 30 min, and protein was measured with (hydrophobic protein) and without (total protein) extraction into chloroform and presented as the weight percent of total BAL phospholipid. Additional thin layer chromatography analysis determined phospholipid class expressed as weight percent relative to large aggregate total phospholipid content. Data are mean ± SEM (median), with n = 6 – 7 rats in each group for the protein analyses and n = 4 for the phospholipid class analyses. Significant differences from a specific group are indicated by

* uninjured

[†] LC, or

[‡] CASP. Adjusting for multiple comparisons, differences were considered significant if $P < 0.0042$ to maintain a family-wise α error less than 0.05.

TABLE 6

Degree of statistical correlation between large surfactant aggregate surface activity and percent content in BAL and lung injury severity parameters

Lung injury parameters	Minimum surface tension at 20 min of bubble pulsation		BAL large aggregate fraction, % total BAL phosphate	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
BAL RBCs	0.820	<0.0001	−0.805	<0.0001
BAL albumin	0.836	<0.0001	−0.819	<0.0001
BAL total protein	0.873	<0.0001	−0.827	<0.0001
BAL protein/phosphate	0.901	<0.0001	−0.872	<0.0001
Lun< MPO	0.884	<0.0001	−0.876	<0.0001
BAL PMNs	0.769	<0.0001	−0.718	<0.0001

Pair-wise correlations and significance values were determined for the indicated parameters of surfactant dysfunction and lung injury severity based on pooled data for uninjured controls, LC at 24, 48, 72, and 96 h, and CASP and LC + CASP at 24 h (n = 39 – 42 for each correlation).

TABLE 7

Degree of statistical correlation between large surfactant aggregate protein and lyso-PC content and lung injury severity parameters

Lung injury parameters	Large aggregate total protein content		Large aggregate lyso-PC content	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
BAL RBCs	0.894	<0.0001	0.847	<0.0001
BAL albumin	0.859	<0.0001	0.821	<0.0001
BAL total protein	0.906	<0.0001	0.894	<0.0001
BAL protein/phosphate	0.918	<0.0001	0.943	<0.0001
Lung MPO	0.763	<0.0001	0.865	<0.0001
BAL PMNs	0.737	<0.0001	0.837	<0.0001

Pair-wise correlations and significance values were determined for the indicated parameters of large aggregate composition and lung injury severity based on pooled data for uninjured controls, LC at 24, 48, 72, and 96 h, and CASP and LC + CASP at 24 h (n = 40 – 43 for each correlation with large aggregate total protein content and n = 26 – 28 for each correlation with lyso-PC content).