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THE EVOLUTION OF ISOLATED BILATERAL LUNG CONTUSION FROM BLUNT CHEST TRAUMA IN RATS: CELLULAR AND CYTOKINE RESPONSES

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Abstract

Lung contusion is the leading cause of death from blunt thoracic trauma in adults, but its mechanistic pathophysiology remains unclear. This study uses a recently developed rat model to investigate the evolution of inflammation and injury in isolated lung contusion. Bilateral lung contusion with minimal cardiac trauma was induced in 54 anesthetized rats by dropping a 0.3-kg hollow cylindrical weight onto a precordial shield (impact energy, 2.45 Joules). Arterial oxygenation, pressure-volume (P-V) mechanics, histology, and levels of erythrocytes, leukocytes, albumin, and inflammatory mediators in bronchoalveolar lavage (BAL) were assessed at 8 min, at 4, 12, 24, and 48 h, and at 7 days after injury. The role of neutrophils in the evolution of inflammatory injury was also specifically studied by depleting these cells with intravenous vinblastine before lung contusion. Arterial oxygenation was severely reduced at 8 min to 24 h postcontusion, but became almost normal by 48 h. Levels of erythrocytes, leukocytes, and albumin in BAL were increased at ≤ 24 h, and returned toward normal by 48 h. Deficits in P-V mechanics were most apparent at 24 h postcontusion. Levels of macrophage inflammatory polypeptide-2, cytokine-induced neutrophil chemoattractant-1, and interleukin 6 in BAL peaked at 24 h, whereas monocyte chemoattractant protein-1 and interleukin 1 β peaked at 24 to 48 h postcontusion. Histology showed early hemorrhagic injury (8 min–12 h), with neutrophilic infiltration at 24 h and areas of bronchiolitis obliterans organizing pneumonia-associated fibrosis at 7 days. Vinblastine-treated neutropenic rats had significantly reduced lung injury based on total lung volume at 4 h and on BAL albumin levels at 24 h postcontusion. Inflammatory injury from isolated bilateral lung contusion in rats is most severe in the acute period (8 min–24 h) after initial blunt trauma, and includes a component of neutrophil-dependent pathology.

Keywords

Lung contusion; blunt trauma; hypoxia; animal model; acute lung injury

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INTRODUCTION

Lung contusion is an important problem in the intensive care of trauma patients. Thoracic trauma is involved in nearly one-third of acute admissions of adults to the hospital (1), and lung contusion is one of the most commonly diagnosed intrathoracic injury resulting from blunt trauma (2,3). Blunt thoracic trauma is also an independent risk factor for the development of pneumonia, clinical acute lung injury, and the acute respiratory distress syndrome, resulting in high mortality rates of 10% to 25% in adults (3,4). The pathophysiology of lung contusion and blunt chest trauma includes ventilation/perfusion mismatching, increased intrapulmonary shunting, increased lung water, segmental lung damage, and a loss of compliance (5). Clinically, patients display hypoxemia, hypercarbia, and increased work of breathing of varying severity and duration (6), which are treated with supplemental oxygen and mechanical ventilation as indicated.

Quantitative clinical-pathological correlations in lung contusion injury are not well defined. There is often little direct correlation between the anatomic extent of contused lung and the degree of hypoxemia (3). Patients with large contusions visible on chest x-ray may have little or no hypoxemia, whereas those with small lung contusions apparent only on computed tomography scanning can be severely hypoxemic. Moreover, the timescale of clinical hypoxia can be relatively brief, or it may be prolonged and progressive. Johnson et al. (7) reported that contusions involving three or more lung lobes were not associated with an increased incidence of ventilatory support or death compared with contusions involving less than three lobes. Also, Erickson et al. (8) found that the volume of lung contusion had no relation to arterial partial pressure of oxygen (PaO₂) in monkeys. A pulmonary contusion score incorporating the number of lobes with contusion has been reported to improve prognostication (9). However, specific cellular and inflammatory factors that contribute to the severity of lung contusion injury and determine its resolution or progression to acute respiratory failure remain poorly understood.

The present study investigates the evolution and resolution of inflammatory injury over 7 days in rats with bilateral lung contusion induced by a weight falling onto a precordial shield to minimize associated cardiac trauma (10,11). The severity of lung injury is assessed by measurements of arterial oxygenation, closed chest pressure-volume (P-V) mechanics, and levels of erythrocytes, leukocytes, albumin, and inflammatory mediators in bronchoalveolar lavage (BAL). Mediators studied include the proximal proinflammatory cytokines tumor necrosis factor (TNF)- α and interleukin (IL)-1 β , the CXC chemokines macrophage inflammatory peptide (MIP)-2 and cytokine-induced neutrophil chemoattractant (CINC)-1, the major mono-tactic chemokine monocyte chemoattractant protein (MCP)-1, and the modulating cytokines IL-6 and IL-10. The specific importance of neutrophils in the evolution of injury was also studied by depleting these cells by intravenous vinblastine before the initiation of lung contusion.

MATERIALS AND METHODS

Induction of isolated lung contusion

All procedures performed on rats were approved by the Institutional Animal Care and Use Committee at the State University of New York (Buffalo, NY), and complied with state and federal regulations, as well as the National Institutes of Health regulations. As previously described, lung contusion was induced in halothane-anesthetized adult male Long-Evans rats (250–300 g body weight; Harlan Sprague-Dawley, Indianapolis, IN) at an energy equivalent of 2.45 J by a hollow cylindrical weight dropped from a defined height onto a precordial shield that minimized associated cardiac trauma (10,11). The path of the falling weight was controlled by a surrounding cylindrical tube and Teflon guides. A total of 54 rats was

studied at times ranging from 8 min to 168 h (7 days) after blunt chest trauma, and an additional nine rats served as uninjured controls for baseline values.

Blood sampling and arterial blood gas measurements

Arterial partial pressure of oxygen (PaO_2) was measured at 8 min, at 4, 12, 24, and 48 h, and at 7 days after injury. At each time point, animals breathed 98% O_2 for 5 min (fraction of inspired oxygen, FiO_2 , 0.98) before blood gas measurements to assess the severity of pulmonary shunting. Halothane (2%) anesthesia was induced and blood (~0.5 mL) was collected from the descending aorta in a heparinized syringe followed by analysis with an ABL5 blood gas analyzer (Radiometer America, Westlake, OH).

Lung P-V mechanics

Pulmonary mechanics were measured immediately after blood samples were collected. A 14-gauge steel cannula was inserted into the trachea of a halothane-anesthetized rat through a 2-cm ventral midline neck incision, and the animal was 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 in-line pressure transducer connected to an Apple PowerBook G4 (Apple Computer, Cupertino, CA) equipped with a data acquisition board (National Instruments, Austin, TX) and software written by the laboratory in Laboratory VIEW 6.0 (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 kilograms of body weight.

BAL and cell counts

After P-V measurements, a midline incision was made through the sternum and the lung vasculature was flushed by injecting 20 mL of Hanks' balanced salt solution at 37°C into the beating right ventricle. BAL was performed by injecting 5×10 mL of 37°C normal saline through the tracheal cannula. Recovered BAL fluid was centrifuged at 1500g at 4°C for 3 min to pellet cells, and the supernatant was frozen for later albumin and cytokine analyses. The cell pellet was resuspended in 4 mL of phosphate-buffered saline (PBS) + 0.1% sodium azide, and total erythrocytes (red blood cells) and leukocytes (white blood cells, WBC) were measured with a Multisizer 3 Coulter Counter (Beckman Coulter, Fullerton, CA). Differential counts were also done on cells after cytocentrifugation (Cytospin 3; Shandon Southern Instruments, Sewickley, PA) and staining with Diff-Quik (Baxter, Detroit, MI).

Albumin concentrations in BAL

Albumin concentrations in BAL were measured by enzyme-linked immunoabsorbant assay (ELISA) with a polyclonal rabbit anti-mouse albumin antibody (generously provided by Dr. Daniel Remick, University of Michigan, Ann Arbor, MI), a horseradish peroxidase-labeled goat anti-rabbit immunoglobulin G (BD Biosciences PharMingen, San Diego, CA), and rat albumin (Sigma, St. Louis, MO) as a standard.

Cytokines and chemokines

Soluble concentrations of $\text{TNF-}\alpha$, $\text{IL-1}\beta$, CINC-1, MIP-2, MCP-1, IL-6, and IL-10 in BAL were determined using ELISA. Antibody pairs (one capture antibody and one biotinylated-reporter antibody) and recombinant cytokines for these assays were obtained from R&D Systems (Minneapolis, MN).

Histopathology

Lung and cardiac tissue samples were obtained from two rats at each time point of injury. All histological examinations were done by an experienced pathologist (J.A.W.) who was blinded to the status of individual animals. Tissue samples for histology were fixed with 1% formalin and were stained with hematoxylin and eosin for standard examination, or with Masson's trichrome to assess the degree of collagen deposition and fibrosis.

Neutrophil depletion with vinblastine

Four days before lung contusion, halothane-anesthetized rats were injected with vinblastine (0.75 mg/kg body weight; Sigma) or an equal volume of PBS as a control via the dorsal penile vein. Circulating neutrophil counts in arterial blood were measured before vinblastine administration, and neutrophil depletion was assessed when animals were sacrificed for injury evaluations (4 and 24 h postcontusion). Adequate neutrophil depletion was prospectively defined as <5 neutrophils per 100 leukocytes in a peripheral smear at the time of injury evaluation. Twelve of 16 rats treated with vinblastine met these criteria and were examined for lung injury based on PaO₂/FiO₂ ratios, albumin levels in BAL, and closed chest inflation/deflation P-V mechanics at 4 and 24 h (n = 6 at each time point). Four rats treated with vinblastine did not meet the prospective limit for neutrophil depletion and were excluded from analysis.

Statistical analyses

Data are expressed as mean ± SEM for a total of n animals per group. One-way analysis of variance (ANOVA) with Dunn's *post hoc* test was used for all intergroup pairwise comparisons. Values for correlations such as *P* value (Fisher's *r*-z test) were ascertained with the Statview software program.

RESULTS

Survival and gross morphology of lung contusion injury

Rats that did not survive (3/54) died immediately after the initial blunt chest trauma (two from intrathoracic hemorrhage in association with lung laceration without identifiable injury to the heart, and one from liver injury and uncontrolled intra-abdominal hemorrhage). Gross morphological assessments in the remaining rats (51/54) at the time of sacrifice indicated that both lungs were superficially affected by contusion in a relatively uniform pattern. Areas of contusion were hemorrhagic at 8 min and 4 h, whereas contused areas were purplish in lungs examined at 24 h. Areas of lung contusion at 48 h were brownish in color, and lungs at 7 days had organized focal contused areas with faint scarring apparent in some cases. All animals demonstrated normal movement, activity, and behavior in the period preceding sacrifice and evaluation at 7 days. As opposed to the lungs, there was minimal evidence of cardiac trauma on gross examination at any time point studied (8 min-7 days).

Histological evolution of lung contusion

Similar patterns of acute lung tissue injury were observed bilaterally on microscopic examination. Lung tissue at 8 min, 4 h, and 12 h postcontusion had diffuse areas of intra-alveolar hemorrhage with disruption of alveoli, along with interstitial hemorrhagic injury that frequently involved perihilar areas and extended to the visceral surface of the pleura. At 24 h, atelectasis was pronounced, and there were increased numbers of leukocytes (predominantly neutrophils) within the alveolar spaces and interstitium. At 48 h, neutrophilic infiltration continued to be prominent, and alveolar lining tissue was thickened and contained abundant fibroblasts. Intra-alveolar edema was also seen at this time (Fig. 1A). At 7 days postcontusion, injury was organized with focal areas of congestion and

intraluminal fibroblastic plugs. Interstitial fibrosis was apparent, particularly around respiratory bronchioles, with microscopic features consistent with bronchiolitis obliterans organizing pneumonia (BOOP), a feature seen in some forms of acute lung injury in humans (12,13) (Fig. 1B). Histological examination of the heart showed some areas of vascular congestion in the walls of the atria (e.g., Fig. 1C at 48 h), but no evidence of traumatic injury to muscle tissue was apparent in any chambers of the heart at any time studied (8 min-7 days). Histological data further documenting the lack of significant trauma to cardiac tissue in this rat model have been reported recently by Raghavendran et al. (10,11).

Hypoxia and alveolar-capillary integrity (albumin leakage) in evolving lung contusion

Arterial oxygenation was examined after a 5-min period of breathing 98% oxygen to assess the severity of pulmonary shunting. Injured rats had severe hypoxia at 8 min after contusion ($\text{PaO}_2/\text{FiO}_2 = 117 \pm 15 \text{ mmHg}$; Fig. 2). Arterial oxygenation improved over time, but values of $\text{PaO}_2/\text{FiO}_2$ were still in the range meeting the definition of clinical acute lung injury ($<300 \text{ mmHg}$) at 4 to 24 h postcontusion. Arterial oxygenation improved significantly by 48 h ($379 \pm 35 \text{ mmHg}$), and there was no statistical difference in $\text{PaO}_2/\text{FiO}_2$ ratios in injured and control rats at 7 days (435 ± 4 vs. $452 \pm 14 \text{ mmHg}$, respectively). Albumin leakage into the airspaces was also maximal early after contusion, peaking at $665 \pm 84 \mu\text{g/mL}$ at 4 h (Fig. 3). Albumin concentrations in BAL remained elevated over 24 h, paralleling deficits in oxygenation. At 7 days, albumin levels in BAL from rats with lung contusion were equivalent to uninjured controls (20.4 ± 1.4 vs. $25 \pm 3 \mu\text{g/mL}$, respectively; Fig. 3).

Cellular content of BAL in evolving lung contusion

Erythrocyte numbers in BAL as determined by multisizer counting peaked by 12 h postcontusion ($2.6 \pm 0.06 \times 10^8$ versus $7.3 \pm 0.1 \times 10^5$ in uninjured controls, $P < 0.001$, Fig. 4A). Erythrocyte numbers returned to baseline in contused rats by 7 days ($3.1 \pm 0.5 \times 10^5$, Fig. 4A). The number of WBC in BAL increased and reached a plateau at 24 h ($3.8 \pm 0.8 \times 10^7$ vs. $8.8 \pm 1.1 \times 10^5$ in uninjured controls, $P = 0.001$, Fig. 4B). The early leukocytic response in the first 24 h after contusion was predominantly neutrophilic (Fig. 4C), and progressed to a more monocytic response at later times (data not shown).

Cytokine profiles in evolving lung contusion

Levels of the neutrophilic CXC chemokines CINC-1 and MIP-2 in BAL peaked at 24 h after lung contusion (Fig. 5, A and B). The concentration of the monocytic chemokine MCP-1 was also increased in BAL at 24 h, and remained elevated at 48 h postcontusion (Fig. 5C). Levels of all three chemokines returned to baseline at 7 days (Fig. 5). Concentrations of IL-6 in BAL were significantly elevated at 24 h postcontusion, and returned to baseline at 7 days (Fig. 6A). In addition, levels of IL-1 β in BAL were elevated at 24 to 48 h postcontusion, and also decreased to baseline at 7 days (Fig. 6B). Concentrations of IL-10 and TNF- α in BAL fluid were at the limits of detection of the assays used at all time points studied (data not shown).

Lung mechanics in evolving contusion injury

Alterations in quasistatic pulmonary P-V mechanics were apparent as early as 8 min postcontusion, when a lower inflection point during inflation was present without a significant change in total lung capacity (Fig. 7). Abnormalities in P-V mechanics were most pronounced at 24 h postcontusion, and included significant decreases in inflation and deflation lung volumes at fixed pressure. For example, at the midpoint pressure of 20 cm H₂O, inflation volumes in rats with lung contusion at 24 h were $12.2 \pm 2.3 \text{ mL/kg}$ versus $22.0 \pm 1.7 \text{ mL/kg}$ for uninjured controls ($P < 0.001$; Fig. 7). Similarly, deflation volumes at the midpoint pressure were $28.5 \pm 2.1 \text{ mL/kg}$ in rats with lung contusion compared with

35.9 ± 0.8 mg/kg in controls at 24 h ($P < 0.001$). Reductions in deflation volumes in rats with lung contusion had largely resolved by 48 h, but P-V inflation volumes were still reduced at this time (Fig. 7). By 7 days postcontusion, inflation and deflation lung volumes in injured rats were identical to those of uninjured controls (Fig. 7).

Effect of neutrophil depletion on lung contusion

To investigate the specific importance of neutrophils in lung contusion injury, rats were treated with intravenous vinblastine (0.75 mg/kg body weight) or PBS as a control at 4 days before lung contusion. Rats treated with vinblastine and evaluated for injury at 4 and 24 h postcontusion met a prospectively defined limit of <5 neutrophils per 100 leukocytes in peripheral blood smears at these times. At 4 h postcontusion, neutropenic and undepleted rats had similar levels of albumin in BAL (826 ± 178 µg/mL and 961 ± 339 µg/mL, respectively, Fig. 8A). However, at 24 h postcontusion, neutropenic rats had significantly reduced BAL albumin concentrations compared with undepleted, injured rats (478 ± 154 µg/mL versus 3601 ± 564 µg/mL, $P < 0.001$, Fig. 8A). BAL albumin levels in neutropenic and undepleted rats with lung contusion were elevated at 4 and 24 h relative to uninjured control rats (25.3 ± 2.6 µg/mL, Fig. 3). Closed-chest quasistatic P-V curves showed that total lung volume at 40 cm H₂O was greater in neutropenic rats compared with undepleted rats at 4 h postcontusion (38.3 ± 1.1 mL/kg versus 29.5 ± 0.1 mL/kg in depleted and undepleted rats, respectively, $P < 0.05$, Fig. 8C). Neutropenic rats also had higher mean lung inflation and deflation volumes at the midpoint pressure of 20 cm H₂O at 4 h postcontusion compared with undepleted rats (Fig. 8C), as well as higher mean PaO₂/FiO₂ ratios at 4 and 24 h postcontusion (Fig. 8B), but these differences did not reach statistical significance.

DISCUSSION

This study has investigated the evolution of inflammatory injury over 7 days after isolated bilateral lung contusion from external blunt chest trauma in rats. Rats had significant arterial hypoxemia and albumin leakage in BAL at 8 min, and at 4, 12, and 24 h postcontusion (Figs. 2 and 3). Erythrocyte numbers in BAL were similarly elevated at ≤24 h postcontusion (Fig. 4), consistent with alveolar hemorrhage in histological assessments (Fig. 1). Quasistatic P-V mechanics were abnormal in the contused lungs, with reductions in inflation/deflation volumes found to be maximal at 24 h (Fig. 7). Levels of MIP-2, CINC-1, and IL-6 in BAL were significantly elevated at 24 h postcontusion, and levels of MCP-1 and IL-1b were increased at 24 and 48 h (Figs. 5 and 6). By 7 days postcontusion, concentrations of all inflammatory mediators in BAL had returned to baseline levels (Figs. 5 and 6), consistent with normalized oxygenation, albumin, cell numbers, P-V mechanics, and histological evidence of fibrosis at this time (Figs. 1–7).

Mechanical factors relevant for lung contusion have been described by Clemmedson and Pettersson (14). These include the “spalling effect” where alveoli and small airways are disrupted by shearing forces, as well as an “inertial effect,” where alveolar tissue is stripped from heavier hilar structures as they accelerate at different rates after impact. Perihilar injury consistent with an inertial effect was observed in our model (Fig. 1). In addition, an “implosion effect” can occur in contusion injury as alveolar and airway tissues rebound from overexpansion of gas after the impact pressure wave passes. Pulmonary contusion in our study likely involved contributions from all these mechanisms. Lungs had bilateral contusions on gross examination, and histology demonstrated acute tissue disruption at the alveolar level (Fig. 1). There was no evidence of substantial traumatic injury to cardiac tissue on gross or microscopic examination at any time studied, consistent with findings reported by Raghavendran et al. (10,11).

Histological assessments showing acute lung contusion injury in our study agreed with changes in $\text{PaO}_2/\text{FiO}_2$ ratios, lung volumes, and levels of albumin, blood cells, and inflammatory mediators in BAL at 8 min to 24 h after the induction of blunt thoracic trauma. Improvements in lung injury based on these parameters at 48 h to 7 days also were generally consistent with histological findings. BOOP-associated fibrosis, which was observed here in rats at 7 days postcontusion, has been reported in other forms of subacute lung injury in humans (12,13). BOOP is characterized by the proliferation of granulation tissue within small airways and alveolar ducts (12). T cells have been implicated as important in BOOP-associated fibrosis (15), and these immune cells may contribute to the pathology and resolution of isolated lung contusion. MCP-1 activation of cells via the CCR2 receptor (an MCP-1 receptor) has also been implicated in the pathogenesis of granulomatous lung disease and bronchiolitis obliterans in lung transplant patients (16).

Reductions in lung volumes and compliance in our experiments were maximal at 24 h postcontusion injury (Fig. 7). These mechanical impairments could indicate the presence of surfactant dysfunction associated with alveolar edema and the movement of albumin into the airspaces, although this was not studied directly in our experiments. Quasistatic pulmonary inflation volumes were severely reduced at 8 min postcontusion, and abnormalities in inflation mechanics persisted at 12 to 24 h (Fig. 7). The presence of very early decreases in lung inflation volumes with a lower point of inflection in the inflation P-V curve suggests that recruitment of alveoli with optimal positive end expiratory pressure (PEEP) might play an important therapeutic role in lung contusion. Early positive pressure ventilation with PEEP has been shown to be beneficial in some patients with lung contusion (17,18). Peak abnormalities in deflation P-V mechanics occurred at 24 h postcontusion (Fig. 7), and were somewhat delayed compared with abnormalities in hypoxemia (maximal at 8 min-4 h, Fig. 2). Relative improvements in arterial oxygenation at 12 to 24 h postcontusion suggest that compensatory mechanisms were becoming active in the injured lungs, such as selective vasoconstriction to shunt blood to better-ventilated portions of the pulmonary parenchyma.

Our experiments found significant increases in the levels of several cytokines and chemokines in BAL from rats with lung contusion (Figs. 5 and 6). Signs of inflammation by 24 h followed by subsequent signs of healing have been noted in humans with lung contusion (19). Neutrophils were prominent in the early leukocytic response (Fig. 4), and their functional importance was studied further by using vinblastine to deplete neutrophils 4 days before the induction of lung contusion. Rats pretreated with vinblastine had significantly lower levels of albumin in BAL at 24 h postcontusion compared with injured, but nondepleted rats (Fig. 8A). Quasistatic lung P-V inflation/deflation curves were also improved in neutrophil-depleted rats, particularly in terms of total lung volume at 4 h (Fig. 8C). These results indicate that acute injury in rats with isolated lung contusion is, in part, neutrophil dependent. However, multiple inflammatory cells and mediators are likely important in the severity and progression of lung contusion injury. For example, elevated levels of MCP-1 were found at 24 to 48 h postcontusion (Fig. 6), and have potential mechanistic significance in the transition from an acute neutrophilic response to a more monocytic subacute pattern of inflammation. Strategies aimed at depleting monocytic rather than neutrophilic cells were not examined here, and this is one area for future investigation.

The finding that isolated bilateral lung contusion has a relatively acute course of evolution and resolution is consistent with the early reversal of hypoxemia observed in many patients diagnosed with this condition. One important speculation from this result is that severe or progressive clinical forms of lung contusion may frequently involve concurrent or secondary insults. We have previously reported that “two-hit” lung injuries in animals can involve substantially more severe pathology than caused by either component insult alone. For example, the severity of aspiration lung injury in rats is significantly increased when two

insults (acid plus small gastric particles) are administered in combination (20,21). It has also been reported that severe lung injury and acute respiratory distress syndrome are more likely in patients if food particulate material is present during gastric aspiration (22,23). In addition, aspiration of acidified milk products can generate increased injury relative to acid or milk products alone (24), and secondary insults like hyperoxia exacerbate acid aspiration lung injury in rats and rabbits (25,26).

In summary, rats with isolated bilateral lung contusion appear to have a self-limiting acute injury that improves by 48 h and is almost fully resolved at 7 days based on measurements of arterial oxygenation, P-V mechanics, and levels of albumin, cells, and inflammatory mediators in BAL. The degree of lung injury at 4 and 24 h was also shown to be neutrophil dependent, in part, based on studies where these leukocytic cells were depleted by administering vinblastine 4 days before contusion. The relatively rapid resolution of isolated lung contusion injury in rats suggests that additional factors such as extrapulmonary trauma or associated insults like gastric aspiration may be important in many patients who develop a more severe and progressive respiratory failure after blunt thoracic trauma.

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Fig. 1. Histopathology of tissue injury in rats with lung contusion

(A) Hematoxylin and eosin-stained lung tissue section at 48 h (10×) showing neutrophils and fluid in the air spaces and thickening of the alveolar lining. (B) Masson's trichrome staining of lung tissue at 7 days postcontusion (40×). Alveolar injury has largely resolved, with repair accompanied by increased collagen deposition around respiratory bronchioles and evidence of fibroblastic plugs (features similar to BOOP, arrowhead). (C) Hematoxylin and eosin-stained sections of the left atrium at 48 h postinjury (10×). Hemorrhagic congestion is present but cardiac muscle is not significantly disrupted. No histological evidence of significant trauma to cardiac muscle was observed at any time point studied (8 min–7 days). See text for details.

Fig. 2. Arterial oxygenation in rats with evolving bilateral lung contusion injury

Arterial oxygenation was assessed as the ratio of PaO₂/FiO₂ (mmHg, mean $\pm$ SEM) in aortic blood after a 5-min period of breathing 98% oxygen. Blood samples were drawn just before sacrifice at 8 min to 168 h (7 days) after induction of lung contusion from a falling weight with a chest impact energy of 2.45 J. Numbers of rats were $n = 9$ at each time point except 12 h ($n = 7$) and 7 days ($n = 8$). The PaO₂/FiO₂ ratio in uninjured control rats was 452 ± 14 mmHg (indicated by the dotted line, $n = 9$). * $P < 0.001$ compared with uninjured controls.

Fig. 3. Albumin concentrations in BAL from rats with evolving lung contusion injury

Albumin levels ($\mu\text{g/ml}$, mean $\pm$ SEM) were measured by ELISA in BAL fluid at the times shown. Numbers of rats were $n = 9$ at each time point except at 12 h ($n = 7$) and 7 days ($n = 8$). The albumin concentration in BAL from uninjured control rats was $25.3 \pm 2.6 \mu\text{g/mL}$ (indicated by the dotted line, $n = 9$). $*P < 0.001$ compared with uninjured controls. See text for details.

Fig. 4. Blood cell numbers in BAL from rats with evolving lung contusion injury (A) Erythrocytes; (B) total leukocytes (WBC); (C) neutrophils. Data are shown at absolute cell numbers (mean $\pm$ SEM), with other details as in the legends to Figures 2 and 3. Cell numbers in BAL from uninjured control rats (dotted lines, $n = 9$) were red blood cells ($7.3 \pm 1.4 \times 10^5$), total WBC ($8.8 \pm 1.2 \times 10^5$), and neutrophils ($9.2 \pm 8.5 \times 10^4$). See text for details. * $P < 0.001$ compare with uninjured controls.

Fig. 5. Concentrations of chemokines in BAL from rats with evolving lung contusion injury (A) MIP-2 (pg/mL); (B) CINC-1 (pg/mL); (C) MCP-1 (pg/mL). Chemokine concentrations were measured by ELISA, with other details the same as in the legends to Figures 2 and 3. Chemokine values for uninjured control rats (dotted lines, $n = 9$) were CINC-1 (13.1 ± 1.5 pg/mL), MIP-2 (undetectable), and MCP-1 (28.0 ± 3.0 pg/mL). * $P < 0.001$ compared with uninjured controls. See text for details.

Fig. 6. Levels of cytokines IL-6 and IL-1 β in BAL from rats after lung contusion (A) IL-6; (B) IL-1 β . Cytokine levels (pg/mL) were measured by ELISA, and other details are as in the legends to Figures 2 and 3. Levels of IL-6 and IL-1 β in uninjured controls (dotted lines, n = 9) were at or below the lower limit of assay detection. * $P < 0.001$ compared with uninjured controls.

Fig. 7. Quasistatic P-V inflation and deflation curves from rats after lung contusion

P-V curves were measured at 8 min, and at 4, 12, 24, 48, and 168 h (7 days) after lung contusion compared with uninjured control rats. Pressures are in centimeters per water and volumes are in milliliters per kilogram of body weight. Points along each curve represent the mean $\pm$ SEM for nine animals at each time point except for 12 h ($n = 8$) and 7 days ($n = 8$). Abnormalities in P-V mechanics compared with uninjured control rats are most apparent at 24 h postcontusion. See text for details.

Fig. 8. Effects of neutrophil depletion on the severity of lung contusion injury

(A) Albumin in BAL; (B) PaO₂/FiO₂ (after breathing 98% O₂ for 5 min); (C) quasistatic P-V curves. Neutrophils were depleted by intravenous Vinblastine, and injury was subsequently evaluated at 4 and 24 h after contusion (see “Materials and Methods”). Neutrophil depletion significantly reduced lung injury based on BAL albumin at 24 h and total lung volume at 4 h as described in the text. Data are mean ± SEM for n = 6 at each time point. #*P* < 0.05, **P* < 0.001 compared with undepleted, contused rats.