Protective Effects of Isoflurane Pretreatment in Endotoxin-induced Lung Injury

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Background: The concept of antiinflammatory effects of volatile anesthetics is well established *in vitro* and in some organ systems. Their protective role in lung injury, however, remains to be elucidated. The authors hypothesized that in the lung, isoflurane pretreatment may attenuate neutrophil infiltration and reduce endotoxin-induced injury.

Methods: Male C57Bl/6 mice were exposed to aerosolized lipopolysaccharide. Neutrophil recruitment into the pulmonary vasculature and migration into the different lung compartments (interstitium and alveolar air space) were determined by flow cytometry. Capillary protein leakage, formation of lung edema, and concentration of the chemokines keratinocyte-derived chemokine (CXCL1) and macrophage inflammatory protein 2 (CXCL2/3) in bronchoalveolar lavage were compared in mice with or without isoflurane treatment (1.4% inspired for 30 min) at different times before and after endotoxin exposure.

Results: Endotoxin inhalation induced significant neutrophil migration into all lung compartments. Isoflurane pretreatment attenuated both neutrophil recruitment into lung interstitium and alveolar space when given 1 or 12 h before or 1 h after lipopolysaccharide but not at 4, 6, or 24 h before endotoxin exposure. Isoflurane pretreatment 1 or 12 h before lipopolysaccharide also reduced protein leakage and pulmonary edema. Production of CXCL1 and CXCL2/3 in the bronchoalveolar lavage was reduced when isoflurane was given 1 h but not 12 h before lipopolysaccharide, suggesting different mechanisms for early and late protection.

Conclusion: Isoflurane pretreatment reduces acute lung injury when given 1 or 12 h before an endotoxin challenge or within the first hour of an already established inflammation.

ACUTE lung injury (ALI) and *acute respiratory distress syndrome* (ARDS) are inflammatory disorders characterized by an excessive infiltration of neutrophils (polymorphonuclear leukocytes [PMNs]), the release of inflammatory mediators, and the destruction of the alveolar–capillary membrane with severe consequences for pulmonary gas exchange.[1] Despite the decrease in mortality over the past decades, there is still no specific therapy beyond mechanical ventilation and other supportive strategies. The molecular mechanisms underlying ALI/ARDS are largely unknown.[2]

Among various stimuli, lipopolysaccharide, a component of the outer membrane of gram-negative bacteria, plays a major role in both the development and the outcome of ARDS.[3] Most forms of lipopolysaccharides bind to Toll-like receptor 4, and signal transduction results in the activation of nuclear factor κB,[4] up-regulation of adhesion molecules,[5] and stimulation of chemokine-induced PMN migration into the lung.[6] In mice, lipopolysaccharide induces the release of the chemokines CXCL1 and CXCL2/3 from alveolar macrophages, type II pneumocytes, and endothelial cells.[7-9] Blocking these chemokines or their common chemokine receptor CXCR2 decreases neutrophil recruitment and lung injury.[10,11]

Polymorphonuclear leukocyte recruitment to inflammatory sites represents a major mechanism of host defense and involves multiple sequential steps including rolling, firm adhesion, and transmigration. In the lung, emigration from the vasculature is followed by transepithelial migration into the alveolar space.[12] Failure of PMN migration into the inflamed lung can increase the severity of pneumonia.[13] Therefore, a controlled modulation of PMN migration is more desirable than a complete blockade.

Isoflurane mediates its anesthetic effects most likely by enhancing inhibitory postsynaptic channel activity and inhibition of excitatory synaptic channel activity. Among others, the γ-aminobutyric acid type A receptor has been demonstrated to play a central role.[14] Other ion channels, including calcium and adenosine triphosphate–sensitive potassium channels, are targeted by isoflurane, and this might be responsible for effects observed beyond anesthesia.

Volatile anesthetics have been shown to exhibit antiinflammatory effects in different organ systems.[15-17] In lung injury, protective effects of pretreatment with volatile anesthetics have not been studied systematically. However, evidence from both *in vitro*[18] and *in vivo*[19] studies suggests that volatile anesthetics might inhibit PMN recruitment by modulating the release of chemotactic cytokines.

In this study, the effect of isoflurane pretreatment on endotoxin-induced lung injury was investigated. Isoflurane was administered before or after lipopolysaccharide exposure, and resulting PMN recruitment into the different lung compartments was assessed. Furthermore, the

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release of the neutrophil chemokines CXCL1 and CXCL2/3 from alveolar space was determined.

Materials and Methods

Mice

Wild-type male C57Bl/6 mice were obtained from Jackson Labs (Bar Harbor, ME). All animal experiments were approved by the Animal Care and Use Committee of the University of Virginia (Charlottesville, Virginia). Mice were aged 8–12 weeks.

Murine Model of Acute Lung Injury

Groups of at least four mice were exposed to aerosolized lipopolysaccharide in a custom-built cylindrical chamber (20 × 9 cm) connected to an air nebulizer (MicroAir; Omron Healthcare, Vernon Hills, IL). The outlet of the chamber was connected to a vacuum pump, and a constant flow rate of 15 ml/min was ensured by a flowmeter (Gilmont Instruments, Barrington, IL). Lipopolysaccharide from *Salmonella enteritidis* (Sigma-Aldrich, St. Louis, MO) was dissolved in 0.9% saline (500 μg/ml), and mice were allowed to inhale lipopolysaccharide for 30 min. As previously shown, this results in a time-dependent PMN recruitment into all compartments of the lung with a peak between 12 and 24 h.[20] In all experiments, control mice were exposed to saline aerosol.

Isoflurane Treatment

Isoflurane was delivered to an identical but separate custom built chamber using an agent-specific vaporizer (Datex-Ohmeda Inc., Madison, WI). Mice were allowed to inhale isoflurane (1.4%) for 30 min.[17] Isoflurane concentration was monitored by a gas-specific analyzer (Capnomac Ultima; Datex, Helsinki, Finland). This isoflurane concentration caused hypnosis, but spontaneous breathing was maintained. Heated pads were used to maintain the animals' temperature. After 30 min, mice were allowed to recover at room air.

Groups of at least four mice each were randomly assigned to receive isoflurane at 1, 4, 6, 12, or 24 h before endotoxin exposure or 1 h after endotoxin exposure (fig. 1). Control mice received no isoflurane.

PMN Recruitment

Polymorphonuclear leukocyte counts in BAL and in lung tissue were determined 12 h after endotoxin exposure as described.[20] Mice were anesthetized with an intraperitoneal injection of ketamine (125 mg/kg; Sanofi Winthrop Pharmaceuticals, New York, NY), xylazine (12.5 mg/kg; Phoenix Scientific, St. Joseph, MO), and atropine sulfate (0.025 mg/kg; Fujisawa, Deerfield, IL). The rib cage was opened through a midline incision, the trachea was cannulated (22-gauge Insyte; Becton Dick-
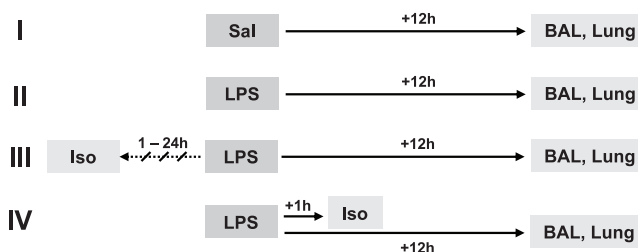
Fig. 1. Experimental protocol for polymorphonuclear leukocyte recruitment. Mice were inhaling saline (Sal; negative control; *I*) or lipopolysaccharide (LPS; positive control; *II*) for 30 min. Twelve hours later, polymorphonuclear leukocyte counts were determined in bronchoalveolar lavage (BAL) and in both compartments of the lung (vasculature and interstitium). Isoflurane pretreatment (Iso; 1.4% for 30 min) was performed 1, 4, 6, 12, or 24 h before lipopolysaccharide stimulation (*III*). A separate group of mice received isoflurane treatment (1.4% for 30 min) 1 h after endotoxin exposure (*IV*).

inson, San Jose, CA), and 5 × 1 ml cold phosphate-buffered saline (PBS) was infused and withdrawn. BAL fluid was centrifuged for 5 min at 300*g*. The pellet was resuspended in 1 ml buffer (1% bovine serum albumin and 0.1% sodium azide in PBS), and a 10-μl aliquot was used for cell count with a hemocytometer. Trypan Blue exclusion was used to determine cell viability.

The fraction of PMNs in BAL was determined by flow cytometry (FACS Calibur; Becton Dickinson, San Jose, CA). PMNs were identified by their typical appearance in the forward scatter/side scatter and their expression of CD45 and 7/4 antigen. Antibodies were purchased from BD Pharmingen (San Diego, CA; anti-CD45; clone 30-F11) and Caltag (Burlingame, CA; 7/4). Isotype controls were used to set the flow cytometry gates.

Lung PMN content in the vasculature and the lung interstitium was determined by a recently developed method to distinguish between PMNs in both compartments.[20] Briefly, 5 min before death, a fluorochrome-labeled antibody (anti GR-1) to murine PMNs was injected intravenously, resulting in a complete labeling of all intravascular PMNs without accessing PMNs in the lung tissue. Anti-mouse GR-1 antibody was purified from supernatant of the GR-1 hybridoma (ATCC) by the biomolecular facility of University of Virginia (Charlottesville, VA). GR-1 was labeled with a staining kit following the manufacturer's protocol (Alexa Fluor 633; Molecular Probes, Carlsbad, CA). Nonadherent PMNs were removed by flushing the pulmonary vasculature through the spontaneously beating right ventricle with 10 ml PBS at a pressure of 25 cm H$_2$O. BAL was performed as described above to remove alveolar PMN. Then, lungs were minced and digested with 125 U/ml collagenase type XI, 60 U/ml hyaluronidase type I-s, and 60 U/ml DNAse1 (all Sigma-Aldrich) at 37°C for 30 min. The presence of excess unlabeled anti–GR-1 prevented possible binding of the injected antibody to extravascular PMNs. A cell suspension was made by passing the digested lungs through a 70-μm cell strainer (BD Falcon,

Bedford, MA). After washing, erythrocytes were lysed, and remaining leukocytes were resuspended and counted. PMNs were then labeled with anti-CD45 and anti-7/4 as described above.

Polymorphonuclear leukocytes in the different lung compartments were assessed in mice receiving lipopolysaccharide without or with isoflurane pretreatment (1 or 12 h before lipopolysaccharide). Control mice inhaled saline.

Capillary Protein Leakage

Pulmonary microvascular permeability was determined using the Evans blue dye extravasation technique. Evans blue (20 mg/kg; Sigma-Aldrich) was injected intravenously 30 min before death. The lungs were perfused free of blood (10 ml PBS through the spontaneously beating ventricle) and removed, and Evans blue was extracted as described previously.[21] The absorption of Evans blue was measured at 620 nm and corrected for the presence of heme pigments: A_{620} (corrected) = A_{620} − $(1.426 \times A_{740} + 0.030)$.[22] Extravasated Evans blue was determined 12 h after lipopolysaccharide or saline inhalation and calculated against a standard curve (micrograms Evans blue dye per gram lung).

Pulmonary Edema

Formation of endotoxin-induced pulmonary edema was determined by wet/dry weight ratios of the lungs.[23] Lungs were removed, blotted dry, and weighed. They were then incubated at 60°C overnight and reweighed. The wet/dry ratio was then calculated.

Histology

Lung histology was performed in three groups of mice: (1) 12 h after saline inhalation, (2) 12 h after lipopolysaccharide inhalation, and (3) isoflurane pretreatment 12 h before lipopolysaccharide inhalation. The pulmonary circulation was flushed with 10 ml PBS and 10 ml paraformaldehyde, 4%, at 25 cm H_2O *via* the spontaneously beating right ventricle. The lungs were subsequently removed and fixed in 80% ethanol for 24 h. Paraffin-embedded sections (5 μm) were prepared and stained with hematoxylin and eosin. Histology sections were reviewed by a blinded observer, and representative areas are presented of each group.

Chemokine Measurements

CXCL1 and CXCL2/3 in the BAL fluid were measured in triplicates using enzyme-linked immunosorbent assay kits, following the procedures supplied by the manufacturer (R&D Systems, Minneapolis, MN). Chemokines were determined in endotoxin-exposed mice with and without isoflurane pretreatment (1 and 12 h before lipopolysaccharide). Saline-exposed mice served as control animals.

Statistics

Statistical analysis was performed with JMP Statistical Software (SAS Institute Inc., Cary, NC). Differences between the groups were evaluated by one-way analysis of variance followed by a *post hoc* Tukey test. Data were presented as mean ± SD, and $P \leq 0.05$ was considered statistically significant.

Results

PMN Recruitment into the BAL

Eight groups of at least four mice each were allocated randomly to receive either saline or lipopolysaccharide with or without isoflurane pretreatment. Lipopolysaccharide inhalation induced significant PMN recruitment into the alveolar air space ($3.9 \pm 0.7 \times 10^6$ PMNs). Almost no PMNs ($3.7 \pm 1.0 \times 10^3$ PMNs) were observed after saline inhalation. Isoflurane pretreatment reduced endotoxin-induced PMN migration into the BAL by approximately 50% when given 1 or 12 h before lipopolysaccharide (1.8 ± 0.3 and $2.1 \pm 0.2 \times 10^6$ PMNs; $P < 0.01$). PMN migration was reduced by approximately 40% when isoflurane was given 1 h after endotoxin exposure ($2.4 \pm 0.8 \times 10^6$ PMNs; $P < 0.05$). No significant differences were observed at other time points (fig. 2A).

PMN Recruitment into the Lung

A flow cytometry–based method was used to distinguish between PMNs in the pulmonary vasculature and in the interstitial space.[20] In control mice inhaling saline, a considerable number of PMNs was found in the pulmonary vasculature ($1.1 \pm 0.3 \times 10^6$), consistent with the concept of a physiologic marginated pool in the lung.[24] However, PMN counts in the interstitial space were negligible. Lipopolysaccharide inhalation led to a significant increase in PMN accumulation in the lung vessels ($2.5 \pm 0.6 \times 10^6$; $P < 0.01$) and interstitial space ($3.4 \pm 1.0 \times 10^6$; $P < 0.001$). When animals received isoflurane 1 or 12 h before lipopolysaccharide, PMN accumulation in the lung vasculature was not affected. However, migration into the lung interstitium was reduced significantly ($1.6 \pm 0.4 \times 10^6$; $P = 0.03$ and $2.2 \pm 0.8 \times 10^6$; $P = 0.03$, respectively; fig. 2B).

Capillary Permeability and Lung Edema

Evans blue extravasation showed that lipopolysaccharide aerosol induced a significant increase in Evans blue leakage into the lung (84 ± 11 *vs.* 33 ± 4 μg/g lung in control animals; $P < 0.01$). Furthermore, formation of lung edema as assessed by wet/dry weight ratio of the lung was increased in endotoxin-treated mice (7.6 ± 1.3 *vs.* 3.9 ± 0.5 in control animals; $P = 0.01$). When pretreated with isoflurane (1 or 12 h before lipopolysaccharide), both capillary leakage and lung edema were

A

B
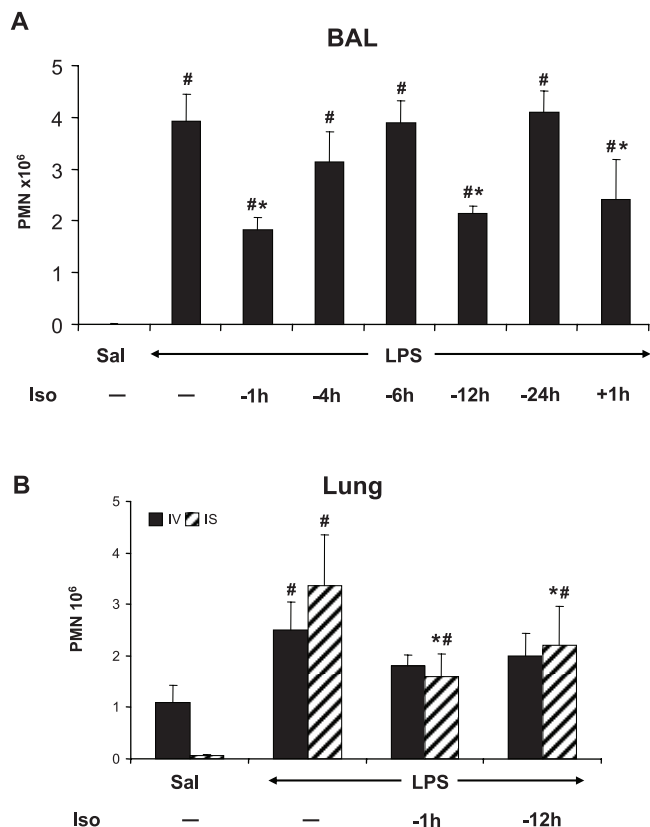
Fig. 2. Polymorphonuclear leukocyte (PMN) recruitment into the bronchoalveolar lavage fluid (BAL; *A*) and into the lung (*B*). Endotoxin-induced PMN influx (#) into BAL was significantly reduced (*) when isoflurane (Iso) was given 1 or 12 h before or 1 h after lipopolysaccharide (LPS). PMNs were present in the vasculature of control lungs (marginated pool; *black bars*), but no interstitial PMNs (*hatched bars*) were observed. Endotoxin induced increased PMN accumulation both in the pulmonary vessels and in the interstitium. PMN counts in the lung interstitium were reduced when animals were pretreated with isoflurane 1 or 12 h before endotoxin exposure. *Graphs* represent mean ± SD from n = 6 (controls, 4 h before and 1 h after lipopolysaccharide) or n = 4 (all other times) animals at each time point. IS = interstitial; IV = intravascular; Sal = saline.

reduced significantly (1 h: 42 ± 7 μg/g lung and 4.5 ± 0.5; 12 h: 55 ± 5 μg/g lung and 5.0 ± 0.9; all $P < 0.05$; fig. 3).
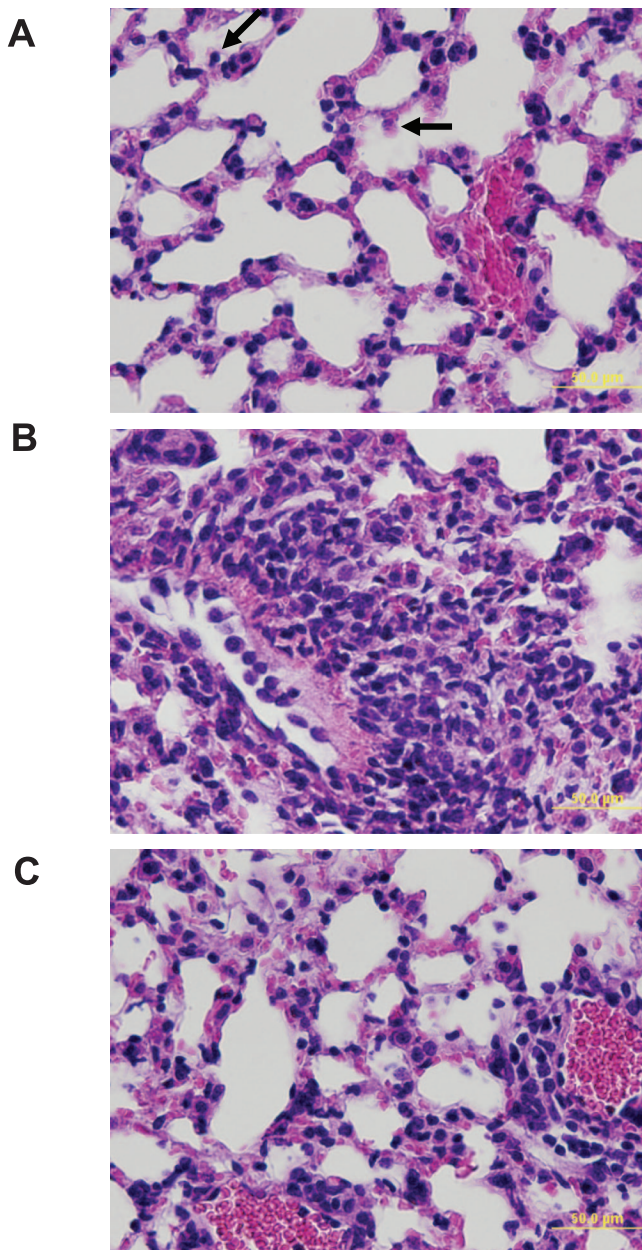
Histology

Lung histology of mice receiving saline inhalation showed a physiologic lung architecture. Alveoli were free of cells except a few (resident) macrophages (fig. 4A, black arrows). Twelve hours after lipopolysaccharide inhalation, the lung was infiltrated by polymorphonuclear and mononuclear leukocytes. The alveolar structure was severely altered (fig. 4B). When mice received isoflurane 12 h before endotoxin exposure, cellular infiltration was reduced (fig. 4C). Furthermore, most leukocytes were found in perivascular locations, suggesting that cell migration was impaired. This supports our quantitative measurements of PMNs in the different compartments in the lung.

A

B
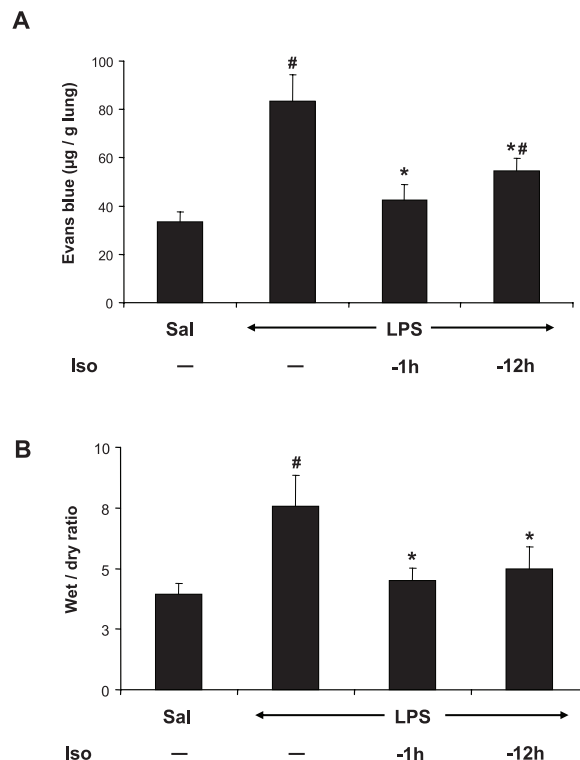
Fig. 3. Capillary protein leakage measured by Evans blue dye extravasation (*A*) and formation of lung edema measured by wet/dry lung weight ratios (*B*) were determined in mice receiving saline (Sal), lipopolysaccharide (LPS), or isoflurane (Iso), 1 or 12 h before lipopolysaccharide. Endotoxin aerosol induced a significant increase in both capillary protein leakage and edema formation, which was reduced by isoflurane pretreatment. Data are mean ± SD from n = 3 animals in each group. # Significant change between control and endotoxin-treated mice. * Significant difference between animals with and without isoflurane treatment.
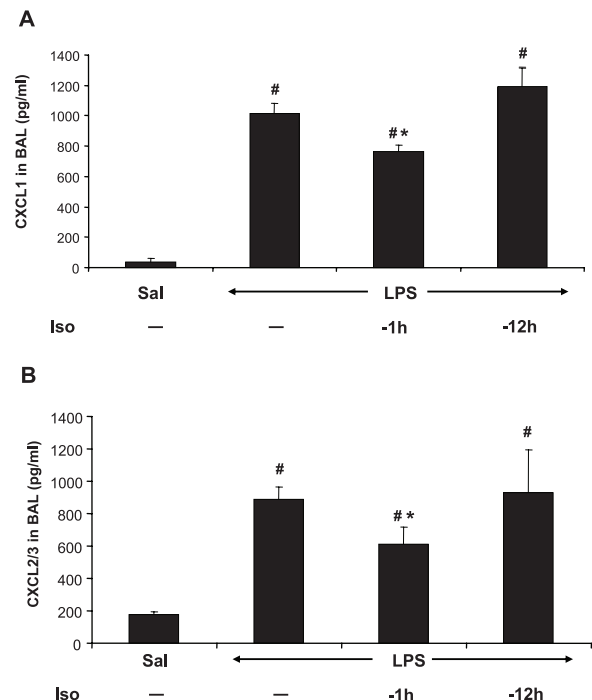
Alveolar Chemokine Release

We used an alveolar stimulus (lipopolysaccharide aerosol) to induce lung inflammation. In this route of application, the release of chemokines from cells in the alveolar compartments such as macrophages or epithelial cells is crucial for PMN recruitment. We therefore measured concentrations of the two most important neutrophil-attracting chemokines, CXCL1 and CXCL2/3, in the BAL fluid from mice that had received saline, lipopolysaccharide, or isoflurane 1 or 12 h before lipopolysaccharide inhalation. Because chemokine production is an early event in response to an inflammatory stimulus, we measured their concentration 2 h after stimulation. Lipopolysaccharide induced a 28-fold increase in CXCL1 (508 ± 33 *vs.* 18 ± 13 pg/ml; $P < 0.0001$) and a 5-fold increase in CXCL2/3 (890 ± 74 *vs.* 182 ± 16 pg/ml; $P < 0.001$). Early isoflurane pretreatment (1 h before lipopolysaccharide) inhibited chemokine secretion markedly (CXCL1: 380 ± 22 pg/ml; $P < 0.005$; CXCL2/3: 609 ± 106 pg/ml; $P < 0.02$). However, chemokine release was not reduced when isoflurane was given 12 h before lipopolysaccharide (fig. 5).

Fig. 4. Lung histology of mice receiving saline inhalation (*A*), lipopolysaccharide aerosol (*B*), or isoflurane 12 h before lipopolysaccharide inhalation (*C*). Resident macrophages (*black arrows*) in *A*. Endotoxin treatment caused massive infiltration of both polymorphonuclear and mononuclear cells (*B*). In mice receiving isoflurane before lipopolysaccharide, the leukocyte infiltration was reduced but perivascular cuffing persists. Representative areas in the different study groups are presented.

Fig. 5. Alveolar release of chemotactic chemokines CXCL1 (*A*) and CXCL2/3 (*B*) was measured by an enzyme-linked immunosorbent assay 2 h after lipopolysaccharide (LPS). Early (1 h before endotoxin exposure) but not late (12 h) isoflurane (Iso) pretreatment reduced CXCL1 and CXCL2/3 secretion significantly. Data are mean ± SD from n = 3 animals in each group. # Significant change between control and endotoxin-treated mice. * Significant difference between animals with and without isoflurane treatment. Sal = saline.

Discussion

In a model of endotoxin-induced lung injury, isoflurane afforded both an early and a late protection from PMN infiltration. Migration was reduced even when isoflurane was administered 1 h after the inflammatory stimulus. Capillary protein leakage and lung edema formation were reduced when mice were pretreated with inhaled isoflurane. We show that early protection (1 h before lipopolysaccharide) is associated with reduced concentration of two critical chemotactic chemokines, CXCL1 and CXCL2/3, in the alveolar space.

Antiinflammatory effects of volatile anesthetics are well established in many organ systems, particularly in the myocardium, where they mediate preconditioning. In the lung, however, protective effects are not well characterized. Giraud *et al.*[19] studied the effect of halothane in a model of endotoxin-induced lung injury. They found reduced PMN recruitment into the BAL and decreased secretion of macrophage inflammatory protein 2 and interleukin 6 when mechanically ventilated rats received halothane over 4 h. Preconditioning of volatile anesthetics was investigated in isolated rat lungs.[25] In this study, pretreatment with either isoflurane or sevoflurane diminished early reperfusion-induced lung edema, vascular permeability, and production of nitric oxide metabolites.

Several mechanisms, most of them focusing on preconditioning, have been suggested to explain the antiinflammatory effects of volatile anesthetics. They include activation of adenosine receptors (most likely A_{2A} and A_3)[26–28] and α- and β-adrenergic receptors, both of which lead to protein C–dependent activation of sarcolemmal and mitochondrial adenosine triphosphate–sensitive potassium channels.[29]

Recent intravital microscopy work has revealed that

isoflurane inhibits endotoxin-induced leukocyte rolling in mesenteric venules.[30] *In vitro* data suggested that isoflurane decreased expression of β_2-integrins on *N*-formylmethionyl-leucyl-phenylalanine-stimulated neutrophils[31] as well as the expression of endothelial adhesion molecules ICAM-1, VCAM-1, and E-selectin.[32] Despite fundamental differences between the systemic and pulmonary microcirculations, PMN recruitment into the lung is known to be dependent on adhesion molecules[33] and chemokines.[11] Altering either or both may influence PMN recruitment.

Nitric oxide derived from inducible nitric oxide synthase (iNOS) has been implicated in acute lung injury. Razavi *et al.*[34] recently demonstrated that, although pulmonary PMN sequestration was attenuated in iNOS$^{-/-}$ mice, transmigration into the BAL was greater in these animals. Furthermore, PMNs from iNOS$^{-/-}$ mice exhibited greater cytokine-induced transendothelial migration when compared with neutrophils from iNOS$^{+/+}$ mice. Volatile anesthetics stimulate nitric oxide production and release, suggesting a potential inhibitory effect on cell migration. In fact, both iNOS[35,36] and endothelial nitric oxide synthase[37] have been shown to participate in isoflurane-induced protection.

Both early and delayed endothelial protection have been demonstrated for isoflurane pretreatment *in vitro*.[38] A similar protective effect on pulmonary endothelium, epithelium, or both might explain the distinct time course observed in our study and is supported by our finding of a blunted permeability increase. We also found that PMN recruitment to the lung interstitium was diminished in isoflurane-pretreated mice, indicating that in our model, volatile anesthetics might have direct effects on the migratory activity of PMNs. This is in line with previous data demonstrating that adhesion decreased when PMNs were incubated with isoflurane or sevoflurane.[39]

Various animal models of ALI/ARDS have been developed to study the molecular mechanisms of neutrophil recruitment.[12] We chose a lipopolysaccharide aerosol challenge because this route results in a massive PMN influx into the BAL comparable with an intratracheal lipopolysaccharide instillation but without the requirement of anesthesia and surgical intervention.[20,40] In our study, early isoflurane protection was associated with decreased production of chemokines CXCL1 and CXCL2/3, both of which are crucial in attracting PMNs in lung injury. Blocking these chemokines or their common receptor CXCR2 almost completely abolished PMN recruitment in different models of pulmonary acute lung injury.[11,23] Volatile anesthetics might, directly or indirectly, inhibit synthesis or secretion of chemokines. Recent work demonstrated that alveolar type II cells in culture exhibited reduced interleukin 1β–induced cytokine secretion when exposed to different volatile anesthetics.[18] The authors demonstrated that interleukin 1β–

induced CXCL2/3 and interleukin-6 secretion in type II alveolar epithelial cells was reduced up to 4 h after halothane exposure on both protein and messenger RNA level. We found that CXCL1 and CXCL2/3 were reduced when isoflurane was given 1 h before lipopolysaccharide, supporting a direct inhibitory effect. However, when isoflurane was given 12 h before lipopolysaccharide, it had no effect on the release of chemokines, suggesting a different protective mechanism.

Clinical Implications

In our study, isoflurane pretreatment was effective at 1 and 12 h before lipopolysaccharide exposure. Isoflurane was able to reduce migration when administered 1 h after the inflammatory stimulus, suggesting a therapeutic role for isoflurane soon after onset of lung injury in addition to its prophylactic benefit, *e.g.*, in lung transplantation.

Although not reflecting all aspects of the human disease, lipopolysaccharide exposure plays a major role in ARDS.[3] In humans, high CXCL8 concentrations in the BAL are associated with increased PMN recruitment[41] and might be a prognostic factor for the development and the outcome of ARDS.[42] Strategies to modulate chemokine-induced PMN recruitment might be attractive in patients prone to or in the early development of ALI/ARDS. However, PMN recruitment is pivotal for the innate host defense. Uncontrolled disruption may have deleterious consequences for patients.[43] In our study, isoflurane pretreatment reduced PMN migration only partially. Moreover, animals were protected from endotoxin-induced capillary leakage and lung edema, suggesting beneficial effects of isoflurane pretreatment.

In vitro data suggest that volatile anesthetics may also exert deleterious effects, including cytotoxicity and inhibition of surfactant production in type II pneumocytes.[44,45] These findings await *in vivo* confirmation. The current study was conducted in spontaneously breathing animals. Mechanical ventilation has been demonstrated to exacerbate inflammatory responses.[46,47] In fact, mechanical ventilation might attenuate or reverse the protective potential of volatile anesthetics.[48] Moreover, preconditioning might also be model dependent. For example, volatile anesthetics have been shown to aggravate acid-induced lung injury.[49] Further investigation to elucidate the molecular targets of volatile anesthetics in the lung will help to find more specific modulators of inflammatory response in ALI/ARDS.

In conclusion, our data demonstrate a significant protective role for isoflurane in ALI, including reduction of endotoxin-induced PMN recruitment and microvascular protein leakage. PMN migration was reduced even when isoflurane was administered 1 h after the inflammatory stimulus. Early but not delayed protection was associated with an inhibition of chemotactic chemokines in the alveolar air space.

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