New Therapeutic Approaches in the Experimental Model

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Background: Acute lung injury caused by gastric aspiration is a frequent occurrence in unconscious patients. Acute respiratory distress syndrome in association with gastric aspiration carries a mortality of up to 30% and accounts for up to 20% of deaths associated with anesthesia. Although the clinical conditions are well known, knowledge about the exact inflammatory mechanisms is still incomplete. This study was performed to define the role of alveolar macrophages in this inflammatory response. In addition, potentially modifying effects of intratracheally applied nuclear factor κB inhibitor pyrrolidine dithiocarbamate were investigated.

Methods: Rat alveolar macrophages were depleted by intratracheal administration of clodronate liposomes, and lung injury was evaluated 6 h after instillation of 0.1N hydrochloric acid. In a second set of experiments, pyrrolidine dithiocarbamate was intratracheally instilled 3 h after hydrochloric acid application, and injury parameters were determined.

Results: Depletion of alveolar macrophages resulted in decreased production of inflammatory mediators in acid aspiration (23–80% reduction of messenger RNA or protein of inflammatory mediators; \( P < 0.05 \)) and consequently also in diminished neutrophil recruitment (36% fewer neutrophils; \( P < 0.01 \)). Treatment with pyrrolidine dithiocarbamate was highly effective in decreasing neutrophil recruitment (66%; \( P < 0.01 \)) and vascular permeability (80%; \( P < 0.001 \)).

Conclusions: These data suggest that alveolar macrophages play an essential role in the inflammatory response of acid-induced lung injury. For the first time, attenuation of acid-induced lung injury with an inhibitor, applied after the onset of injury, is shown.

ACID-INDUCED lung injury is a useful experimental system for the investigation and characterization of immunopathogenic changes and mechanisms in acute lung injury of the corresponding clinical event, the pulmonary acid aspiration.1–5 Aspiration is well known as a severe complication of anesthesia.4,5 It is still a major cause of acute respiratory distress syndrome.6,7

The interaction of effector cells (activated phagocytes = neutrophils and alveolar macrophages [AMs]) with target cells such as epithelial cells in the respiratory compartment of the lung is a well-known and important step in the inflammatory cascade. One of the primary sources of tissue injury is phagocyte-derived substances, such as reactive oxygen metabolites, nitric oxide, and proteases, but indirectly also inflammatory mediators, recruiting effector cells.8 Phagocytic cells can be directly or indirectly activated by chemoattractants and cytokines as well as bacterial lipopolysaccharides or gastric acid. Interaction of any of these stimuli with specific receptors on the cell surface activates these cells. In response to this activation, cells generate cytokines (tumor necrosis factor α [TNF-α]), chemokines (monocyte chemoattractant protein 1 [MCP-1], macrophage inflammatory protein 1β [MIP-1β], cytokine-induced neutrophil chemoattractant 1 [CINC-1], and macrophage inflammatory protein 2 [MIP-2]), and oxygen metabolites. At the same time, they start phagocytizing particles as well as secreting cytoplasmic granules.9 The role of neutrophils in acid aspiration has been elucidated in neutropenic animals, which showed lower endothelial permeability compared with control animals with acid aspiration and circulating neutrophils.1 However, no data exist about the role of AMs in this model of injury.

Many efforts have already been made to exactly define inflammatory processes in aspiration pneumonitis, using animal models of acid aspiration. As previously shown by others and our group, acid-induced lung injury is a biphasic inflammatory process. Kennedy et al.10 showed two peaks of increased permeability, and a biphasic expression pattern of inflammatory mediators and a biphasic recruitment of polymorphonuclear cells was demonstrated by Madjdpour et al.11 A first peak in the accumulation of neutrophils is seen at 0.5–1 h after injury, followed by a second peak occurring 2 h later. The biphasic inflammatory reaction upon aspiration is of special interest because it offers a potential window of therapeutic intervention after the first peak of inflammation.

The expression of inducible genes leading to the formation of the proteins relies on transcription factors. The transcription factor nuclear factor κB (NF-κB) plays a central role in the regulation of many genes, including genes coding for cytokines, chemokines, and adhesion molecules (intercellular adhesion molecule 1 [ICAM-1]).12–14 As previously elucidated, intratracheally applied pyrrolidine dithiocarbamate (PDTC), an NF-κB inhibitor,
attenuates acid-induced lung injury when given before injury. However, no data exist about PDTC treatment after the onset of an injury, which carries even more clinical relevance.

A first aim of this study was to evaluate the role of AMs as effector cells in the inflammatory process of acid aspiration. We hypothesized that AMs, although quantitatively not a predominant cell type, might play a major role in the production of inflammatory mediators and neutrophil recruitment. In a second experimental step, the antiinflammatory effect of PDTC, intratracheally applied after the first peak of inflammation, was explored under the hypothesis that PDTC could attenuate the inflammatory response.

Materials and Methods

Animals

Specific pathogen-free male Wistar rats (250–300 g) were purchased from Janvier (Le Genest-St. Isle, France). Rats were anesthetized with subcutaneously administered 0.25 ml/kg body weight Hypnorm® (fentanyl–fluanisone; Janssen, Beerse, Belgium), 0.25 ml/kg body weight Domitor® (medetomidine; Pfizer, Inc., Westchester, PA), and 0.05 ml/kg body weight atropine, 0.1%. All animal experiments and animal care were approved by the Swiss Veterinary Health Authorities.

Rat Gastric Aspiration Model

Anesthesia was induced in rats, and a midline incision was made to expose the trachea, into which 1 ml/kg endotoxin-free acidic solution (0.1N, pH 1; Sigma, Buchs, Switzerland) was applied. For control animals, phosphate-buffered saline (PBS) was used.

For experiments with intratracheal application of PDTC, 1 ml/kg PDTC, 25 μM, was used 3 h after the onset of injury. Control animals received PBS.

Alveolar Macrophage Depletion

Clodronate liposomes were prepared as previously described. Briefly, liposomes composed of soy phosphatidylcholine (880 mg), cholesterol (132 mg), and DL-α-tocopherol (5 mg) were added to a clodronate solution (375 mg clodronate in 10 ml; Ostac®; Boehringer, Mannheim, Germany) by freeze-thawing and filter extrusion. Unencapsulated clodronate was removed with an Amicon ultrafiltration cell, followed by size exclusion chromatography on a Sephadex G25 column (Pharmacia, Uppsala, Sweden). For the application of liposomes, animals were anesthetized and placed in a supine position. The trachea was exposed surgically, and a 25-gauge needle was inserted into the trachea. Control liposomes or liposomes containing 500 μg clodronate were injected in a volume of 300 μl into the lungs. Depletion rate of AMs after 72 h varied between 70 and 80%. Control animals received liposomes without clodronate.

Bronchoalveolar Lavage

Rats were exsanguinated at predefined time points. The vascular system was flushed and bronchoalveolar lavage was performed with 10 ml PBS as described previously. Bronchoalveolar lavage fluid (BALF) was centrifuged, and cells were analyzed using cytospins and Diff-Quick (Dade Behring, Düdigen, Switzerland).

Lung Tissue Myeloperoxidase

Lungs were homogenized in a buffer containing 50 mM potassium phosphate, 0.5% hexadecyltrimethylammonium bromide, and 5 mM EDTA, sonicated and centrifuged as described previously. Supernatant (50 μl) was added to 1,450 μl assay buffer, consisting of 100 mM potassium phosphate, o-dianisidine hydrochloride, and 30% H2O2. The reaction was assayed every 10 s at 420 nm (enzyme-linked immunosorbent assay [ELISA] reader [Bioconcept, Allschwil, Switzerland]). The results are shown as the slope of change in optical density over 360 s. Control values were defined as 1, and results from stimulated lungs were normalized to the value of 1.

Reverse Transcription-polymerase Chain Reaction for TNF-α, ICAM-1, MCP-1, MIP-1β, CINC-1, and MIP-2 mRNA

Total RNA was extracted from previously flushed and lavaged lungs using Trizol® (Life Technologies, Basel, Switzerland) according to the manufacturer’s protocol. Total RNA (5 μg) was reverse transcribed, and polymerase chain reaction was performed with primers designed for rat TNF-α, ICAM-1, MCP-1, and MIP-1β as seen in table 1. The polymerase chain reaction product was confirmed by electrophoresis in a 1.2% agarose gel.

ELISA Quantification of MCP-1, CINC-1, and MIP-2 in BALF of Rat Lungs

Monocyte chemoattractant protein 1 was assessed in BALF of control and hydrochloric acid (HCl)-exposed animals without or with AMs using standard ELISAs purchased from BD Biosciences (San Diego, CA), and CINC-1 and MIP-2 were assessed with an ELISA purchased from R&D Systems (Abingdon, United Kingdom). The minimum detectable concentration of MCP-1 was 62 pg/ml, that of CINC-1 was 15.6 pg/ml, and that of MIP-2 was 31.2 pg/ml.

Western Blot Analysis of MIP-1β in BALF of Rat Lungs

Bronchoalveolar lavage fluid was loaded and electrophoresed in a 12.5% sodium dodecylsulphate-polyacrylamide gel. After separation, the proteins were blotted to a nitrocellulose membrane for 2 h at 200 mA (BioRad, Hercules, CA). Equal loading of proteins was confirmed by Ponceau S staining. The blot was washed in PBS and blocked with PBS–4% low-fat milk–0.1% Tween-20 for 1 h at room temperature, followed by an
overnight incubation with a polyclonal rabbit anti-rat MIP-1 antibody, 1:100 (vol/vol) (Biovision, Lausen, Switzerland), diluted in blocking buffer. All washing steps were performed three times with PBS–0.1% Tween-20. A secondary horseradish peroxidase–labeled anti-rabbit immunoglobulin G (1:5,000, vol/vol) in blocking buffer was added for 1 h at room temperature. Signals were detected by enhanced chemiluminescence.

**Albumin ELISA**

For detection of albumin in BALF, a sandwich ELISA was developed as previously described and modified.16,18 Briefly, a coating carbonate buffer (0.1 M carbonate, pH 9.5) was used to dilute samples (1:1,000, vol/vol), and a standard curve was created using recombinant rat albumin (RDI, Flanders, NJ). A 96-well plate was coated with 100 μl/well (Biovision, Lausen, Switzerland), diluted in blocking buffer. All washing steps were performed three times with PBS–0.1% Tween-20. A secondary horseradish peroxidase–labeled anti-rabbit immunoglobulin G (1:5,000, vol/vol) in blocking buffer was added for 1 h at room temperature. Signals were detected by enhanced chemiluminescence.

**Hemoglobin Content**

To assess lung injury by quantification of pulmonary hemorrhage, the change in hemoglobin content in BALF was determined according to a modified protocol described previously.19,20 Erythrocytes in the cell pellet of bronchoalveolar lavage were lysed with distilled water for 30 s, and the reaction was then stopped with 2.7% NaCl. Absorption was read at 540 nm.

**Statistical Analysis**

All experiments were performed at least three times. The exact number of rats in each group was five. Results are expressed as mean ± SEM, and the t-test (unpaired, two-tailed) at a 5% significance level was used to determine statistical significance of the differences between means.

**Results**

**Alveolar Macrophage Depletion Studies**

**Cell Recruitment.** Normal rats, which were not treated with liposomes, showed a mean cell content of 1.6 × 10⁶ cells (all macrophages) in BALF. Seventy-two hours after liposome instillation, the mean cell count in BALF of control liposome animals was 2.2 × 10⁶ cells (all macrophages), whereas BALF of clodronate liposome–pretreated animals had an average of 1.0 × 10⁶ cells (0.5 × 10⁶ macrophages and 0.5 × 10⁶ neutrophils). The mild neutrophilic response to clodronate is a known observation.21

Before experiments with clodronate liposomes were initiated, different depletion conditions were evaluated as described.16 Optimal depletion was seen at a dose of
500 μg for 72 h. Depletion was verified with macrophage staining in BALF demonstrating a depletion of 70–80%, which was consistent with previous reports.22

Interstitial neutrophil recruitment upon acid aspiration after 6 h of injury is shown in figure 1A. In animals with AMs, neutrophil content increased after HCl instillation by 431% (P < 0.0001). Without AMs, however, this increase was only 97% (P < 0.0001). Eighty-two percent fewer interstitial neutrophils were found in HCl animals compared with HCl animals with AMs (P < 0.0001).

Instillation of acid was associated with an increase in the number of effector cells (neutrophils and AMs) in BALF as well (PBS animals with 4.5 × 10⁶ cells/ml, HCl animals with 21 × 10⁶ cells/ml). Upon macrophage depletion, this increase was reduced to 12 × 10⁶ cells/ml (P < 0.05). Thirty-eight percent fewer cells (AMs and neutrophils) were seen in BALF compared with undepleted HCl animals (P < 0.05; fig. 1B). Neutrophil content in BALF of HCl-exposed lungs decreased by 56% in AM-depleted animals compared with control liposome–HCl animals (P < 0.05).

Production of Inflammatory Mediators.

Whole Lung. Messenger RNA (mRNA) of whole lung inflammatory mediators was up-regulated after HCl instillation by the following magnitudes (fig. 2): TNF-α, 111% (P < 0.01); ICAM-1, 91% (P < 0.01); MCP-1, 256% (P < 0.01); MIP-1β, 129% (P < 0.05); CINC-1, 160% (P < 0.001); and MIP-2, 140% (P < 0.05). Notably, HCl animals without AMs presented with decreased expression of inflammatory mediators compared with HCl animals with AMs (TNF-α, 62% less, P < 0.01; fig. 2A; ICAM-1, 64% less, P < 0.01, fig. 2B; MCP-1, 64% less, P < 0.05, fig. 2C; MIP-1β, 35% less, P < 0.01, fig. 2D; CINC-1, 23% less, P < 0.05, fig. 2E; and MIP-2, 80% less, P < 0.01, fig. 2F).

Bronchoalveolar Lavage Fluid. Enhanced production of MCP-1 (by a factor of 50) was seen in BALF of animals with acid instillation (increase from 100 to 4,994 pg/ml; P < 0.0001; fig. 3A). Upon AM depletion, MCP-1 production was reduced by 55% in comparison with HCl animals with AMs (P < 0.001). A similar result was found with MIP-1β (fig. 3B): MIP-1β concentration had increased by 162% (P < 0.0001) in HCl compared with PBS animals. HCl animals without AMs had 44% less MIP-1β than HCl-stimulated animals with AMs (P < 0.01). The concentration of the neutrophil chemoattractants CINC-1 and MIP-2 increased by 611% (P < 0.001) and 929% (P < 0.0001), respectively. AM depletion led to 41% less CINC-1 (P < 0.01; fig. 3C) and 26% less MIP-2 (P < 0.05; fig. 3D).

Vascular Permeability and Hemorrhage. The measurement of albumin in BALF is an indirect method for the determination of vascular leakage (fig. 4). Albumin concentration increased 15-fold after HCl instillation, from 116 to 1,727 ng/ml (P < 0.001). Comparing HCl animals with and without AMs, no statistically significant difference in permeability was observed.

Detection of hemoglobin in BALF is a sensitive sign for lung hemorrhage. There was no difference in hemoglobin content in acid-stimulated lungs with or without AMs (data not shown).

Studies with PDTC Intervention

Cell Recruitment. Instillation of PDTC resulted in a decrease in myeloperoxidase activity by 37% in HCl animals compared with the HCl–PBS group (P < 0.01; fig. 5A).

A decrease of effector cell count was also found in BALF of the PDTC-treated HCl group compared with HCl–PBS animals (58% fewer effector cells; P < 0.01; fig. 5B). Upon PDTC intervention, neutrophil recruitment into the respiratory compartment was diminished by 66% (P < 0.05).

Production of Inflammatory Mediators.

Whole Lung. When PDTC was administered intratracheally after the first peak of inflammation, a significantly
different expression pattern of inflammatory mediators was observed (fig. 6). mRNA for whole lung TNF-α decreased by 36% ($P < 0.01$; fig. 6A), that of ICAM-1 decreased by 60% ($P < 0.01$; fig. 6B), that of MCP-1 decreased by 39% ($P < 0.01$; fig. 6C), that of MIP-1β decreased by 43% ($P < 0.01$; fig. 6D), that of CINC-1 decreased by 20% ($P < 0.05$; fig. 6E), and that of MIP-2 decreased by 17% ($P < 0.05$; fig. 6F).

**Bronchoalveolar Lavage Fluid.** To verify production of inflammatory mediators in the respiratory compartment, MCP-1, MIP-1β, CINC-1, and MIP-2 concentrations were determined in BALF. MCP-1 protein was reduced by 55% in BALF of PDTC-treated HCl animals compared with HCl–PBS animals ($P < 0.01$; fig. 7A). MIP-1β was decreased by 51% in acid-injured lungs after PDTC intervention ($P < 0.05$; fig. 7B). The expression of the CXC chemokine CINC-1 was not significantly different in the PDTC-treated group compared with the control group (fig. 7C). MIP-2, however, was decreased by 75% ($P < 0.005$; fig. 7D).
Permeability and Hemorrhage. The leakage of albumin from the vascular into the respiratory compartment was significantly reduced after PDTC application (fig. 8A). BALF of HCl–PBS animals showed an albumin concentration of 1,825 ng/ml, whereas 80% less protein was detected in HCl–PDTC animals (P < 0.001).

Hemoglobin content in acid-injured lungs increased by 138% (P < 0.005) compared with control lungs (fig. 8B). Intervention with PDTC decreased intraalveolar hemoglobin by 58% (P < 0.05).

Discussion

A main objective of this study was to indirectly quantify the role of AMs. In a second set of experiments, the effect of the NF-κB inhibitor PDTC, applied intratracheally after the onset of the inflammatory cascade, was evaluated. Our data show that AMs play a pivotal role in acid-induced lung injury, although as previously demonstrated, neutrophils are quantitatively the predominant effector cell type in this lung injury model. PDTC seems to be an effective blocker of the inflammatory reaction, even when applied after initiation of the injury.

Aspiration of gastric contents has been associated with acute lung injury characterized by pulmonary edema, severely diminished gas exchange, and progression to acute respiratory distress syndrome. A distinct characteristic of this lung injury pattern has been neutrophil infiltration into the lungs. However, the first line of cellular defense for the lower respiratory tract is AMs. They are pivotal effector cells of protective immunity because of their phagocytic activity and their ability to release cytokines, chemokines, and bactericidal products, including proteases. It has been shown in several lung injury models that activated pulmonary macrophages secrete the cytokine TNF-α as well as the chemokines MCP-1, MIP-1β, CINC-1, and MIP-2. These pivotal cytokines and chemokines, together with adhesion molecules, play a crucial role in the orchestration of an inflammatory response, particularly in neutrophil recruitment. A possible relevance of enhanced expression of these inflammatory mediators could be shown in a large number of inflammatory and antigen-induced models of lung diseases such as asthma, reperfusion-induced
lung injury, lung damage caused by immunocomplexes, and endotoxin-induced lung injury.\textsuperscript{31–34}

Macrophage depletion by intratracheal application of dichloromethylene diphosphonate (Cl\textsubscript{2}MDP) liposomes (clodronate liposomes) as phagolysosomes is a well-established method.\textsuperscript{35} Phagocytosis of clodronate liposomes has been shown to result in the selective elimination of macrophages at a rate of up to 80%. However, it is known that a mild accumulation of neutrophils is directly induced by this procedure.\textsuperscript{21} This observation was also confirmed in our study. In addition, comparing data from the depletion study with data from PDTC experiments regarding polymorphonuclear cell recruitment, it is obvious that the number of cells in BALF was much higher in the liposome study. A potential contamination of liposomes with endotoxin was excluded using a Limulus test. Application of liposomes in this model might slightly trigger a following injury, although the mechanisms are not clear.

Relatively little is known about the function of AMs in acid aspiration. A recent study showed that AMs recovered after acid instillation produced TNF-\(\alpha\) and nitric oxide \textit{in vitro}.\textsuperscript{36} In endotoxin-induced lung injury, increased concentrations of whole-lung TNF-\(\alpha\) are present after 30 min of injury, peaking within 6 h as shown by Xing \textit{et al.}.\textsuperscript{37} This group hypothesized that at early time points in endotoxin-induced injury, AMs are the major source of TNF-\(\alpha\), whereas at later time points, the main TNF-\(\alpha\) source is represented by neutrophils. This could be confirmed in our study by showing whole-lung TNF-\(\alpha\) to be macrophage dependent at an early time point of injury because depletion of AMs led to significantly decreased expression of TNF-\(\alpha\) mRNA.

Previous studies have furthermore shown that the chemotactrant MIP-1\(\beta\) might be involved in neutrophil recruitment in acute lung injury.\textsuperscript{30} At 6 h of acid-induced injury, AM-secreted MIP-1\(\beta\) could represent a possible factor for neutrophil accumulation because MIP-1\(\beta\) concentration was decreased in AM-depleted acid-injured animals. However, because CXC chemokines such as CINC-1 and MIP-2 are more important in recruiting neutrophils, we determined these mediators. Our findings provide evidence that AMs secrete CINC-1 and MIP-2 upon stimulation with acid and therefore could be involved in attracting neutrophils as main chemoattractants.

Monocyte chemoattractant protein 1 has recently been shown to play a pivotal role not only in monocyte but also in polymorphonuclear cell recruitment in various experimental systems.\textsuperscript{38,39} MCP-1 was shown to be an important factor for neutrophil recruitment in hyperoxia-exposed rat lungs and in the pathogenesis of tuberculosis.\textsuperscript{40,41} Studies in a mouse model of lipopolysaccharide-induced lung injury showed increased recruitment of neutrophils upon instillation of both lipopolysaccharide and recombinant MCP-1 into the airways.\textsuperscript{42} Our studies support the hypothesis of decreased neutrophil recruitment upon AM depletion with decreased concentrations of MCP-1.
The current in vivo studies demonstrate the important role of AMs in acid aspiration-induced lung injury by either directly or indirectly secreting inflammatory mediators such as TNF-α, MCP-1, MIP-1β, CINC-1, and MIP-2. These chemoattractants can be assumed to play a proinflammatory role in the acid-induced inflammatory orchestration. Similar results with AM-dependent production of inflammatory mediators were found previously in an animal model of hypoxia-induced lung injury. A recent study demonstrated that depletion of AMs in a murine model of Klebsiella pneumoniae induced lung injury decreased survival dramatically by causing 100% lethality 3 days after infection, thereby demonstrating that AMs exert a crucial role in this model. In contrast to these findings, the elimination of AMs promoted pulmonary immunity in a model of Listeria-induced lung injury.

Animal models of acid instillation into the lung allow for the systematic investigation of the processes and mechanisms of acid aspiration and are likely to contrib-

Fig. 6. Determination of whole-lung messenger RNA of tumor necrosis factor 1α (TNF-1α; A), intercellular adhesion molecule 1 (ICAM-1; B), monocyte chemoattractant protein 1 (MCP-1; C), macrophage inflammatory protein 1β (MIP-1β; D), cytokine-induced neutrophil chemoattractant 1 (CINC-1; E), and macrophage inflammatory protein 2 (MIP-2; F). Phosphate-buffered saline (PBS) or 0.1 N acidic solution (HCl), pH 1, was instilled intratracheally. Three hours after the onset of lung injury, pyrrolidine dithiocarbamate (PDTC) or PBS as a control was instilled intratracheally instilled, and the lungs were analyzed 6 h after the onset of injury. Whole-lung RNA was extracted, and reverse-transcription polymerase chain reaction was performed. Equal loading was shown with 18S bands (G). Densitometric values are expressed as percentage of values obtained from PBS + PBS animals (= 1) and are shown as mean ± SEM from five different assays (panels at right). White bars represent values from control animals, and black bars represent values from animals after HCl stimulation. The results of two blots are shown.
ute to a greater understanding of the relevant pathophysiology to ultimately help improve treatment. We have recently reported that acid-induced lung injury in rats is associated with a biphasic inflammatory reaction with neutrophil recruitment with a first peak at 1 h followed by a second one 6–8 h later. The biphasic character of this model is unique and therefore very promising regarding a potential effective drug treatment. Many attempts have been made to reduce acid-induced lung injury. In several studies, acid-induced lung injury has been reduced by either blocking neutrophil products or inhibiting neutrophil migration into the alveoli.1,10,26,27,44–47 Kudoh et al.48 demonstrated an attenuation of acid-induced injury with the administration of pentoxifylline. Furthermore, multiple cytokine release blockers inhibited the production of inflammatory cytokines and attenuated acid-induced lung injury.49 However, all of these mentioned studies focused on an intervention before the onset of the injury, which represents a rather theoretical approach. Our study elucidates for the first time a post hoc therapeutic intervention, which is clinically more relevant. These first results are very promising because—in contrast to other studies—they directly translate into a clinical situation.

Nuclear factor κB is an important transcriptional factor in numerous inflammatory reactions as shown in previous studies.14 Binding of NF-κB to the respective sequence on genomic DNA for inflammatory mediators such as cytokines or chemokines results in a rapid and effective transcription of these genes. PDTC thereby inhibits the activation of NF-κB. Several studies have already been performed applying NF-κB inhibitors and evaluating an antiinflammatory effect.50 Nathens et al. showed an attenuation of endotoxin-induced lung injury in rats after intraperitoneal instillation of PDTC.51 Also in a model of multiorgan failure, the application of PDTC led to a decrease of injury.52 These studies demonstrated
the antiinflammatory effect of PDTC but lacked a local targeted effect. Our results, however, were achieved by intratracheal application of this NF-kB inhibitor, thus avoiding or reducing systemic effects.

In summary, these results suggest that AMs might play a key role in mediating acid-induced lung injury. In addition, a new way of attenuating acid-induced lung injury was found by intratracheal application of a NF-kB inhibitor after the onset of the injury. These findings warrant the development of further strategies to prevent or therapeutically modify acid-induced inflammatory reactions.

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