Conebulization of surfactant and urokinase restores gas exchange in perfused lungs with alveolar fibrin formation

RALPH THEO SCHERMULY,1 ANDREAS GÜNTHER,1 MONIKA ERMERT,2 LEANDER ERMERT,3 HOSSEIN ARDESCHIR GHOFRANI,1 NORBERT WEISSMANN,1 FRIEDRICH GRIMMINGER,1 WERNER SEEGER,1 AND DIETER WALMRATH1

Departments of 1Internal Medicine, 2Anatomy, and 3Pathology, Justus-Liebig-University Giessen, D-35392 Giessen, Germany

Received 14 September 2000; accepted in final form 6 November 2000

Conebulization of surfactant and urokinase restores gas exchange in perfused lungs with alveolar fibrin formation. Am J Physiol Lung Cell Mol Physiol 280: L792–L800, 2001.—Alveolar fibrin generation has been suggested to possess strong surfactant-inhibitory potency. In perfused rabbit lungs, fibrin formation in the alveolar space was induced by sequential ultrasonic aerosolization of fibrinogen and thrombin, and the efficacy of rescue administration of surfactant and urokinase was investigated.

Ventilation-perfusion (V/Q) distribution was assessed by the multiple inert gas elimination technique. Aerosolization of fibrinogen (∼20 mg/kg body wt) increased shunt flow to ∼7%. Sequential nebulization of fibrinogen and thrombin (1.3 U/kg body wt) caused alveolar fibrin deposition, documented immunohistologically, and provoked marked shunt flow, progressing to ∼22% at the end of the experiments. The hemodynamics were virtually unchanged. Rescue aerosolization of natural bovine surfactant (15 mg/kg body wt) or urokinase-type plasminogen activator (4,500 U/kg body wt), undertaken after fibrin formation, improved gas exchange but progressive shunt flow still occurred (efficacy, surfactant > urokinase). In contrast, conebulization of surfactant and urokinase reversed shunt flow to ∼7%, with an increased appearance of normal V/Q matching. We conclude that alveolar fibrin formation is a potent surfactant-inhibitory mechanism in intact lungs, provoking severe V/Q mismatch with a predominance of shunt flow, and that rescue aerosolization of surfactant plus urokinase may offer restoration of gas exchange under these conditions.

urokinase-type plasminogen activator; thrombin; fibrinogen; shunt flow; aerosolization; ventilation-perfusion ratio

ALVEOLAR FIBRIN FORMATION is a hallmark of many inflammatory lung diseases including acute respiratory distress syndrome (ARDS) (13, 16). Under these conditions, both a manifold increased leakage of plasma proteins, including fibrinogen, across a leaky endothelial and epithelial barrier and a pronounced shift of the alveolar hemostatic balance toward the procoagulative side have been documented (1, 10, 15, 16). Alveolar protein levels may be elevated >10-fold in ARDS lungs, and the procoagulant activity, attributable to tissue factor and factor VII, was noted to be increased by approximately two orders of magnitude in severe ARDS and pneumonia. Both alveolar macrophages and epithelial cells have been shown to express and shed tissue factor into the alveolar lining layer in response to inflammatory stimuli (3, 14). Moreover, alveolar levels of the urokinase-type plasminogen activator (uPA), the predominant plasminogen activator of the bronchoalveolar compartment, were found to be depressed in ARDS lungs (1, 10, 16), whereas plasminogen activator inhibitor-1 levels were highly increased under these conditions (4, 15, 21). Both fibrinogen leakage and these alterations in the alveolar hemostatic balance may thus be assumed to underlie alveolar fibrin formation in ARDS and other acute inflammatory lung diseases.

Pulmonary surfactant is a lipoprotein complex covering the alveolar surface and reducing the alveolar surface tension to near zero values at end expiration. Thereby, atelectasis during end expiration is prevented, and breathing is made feasible under regular transthoracic pressures. Impaired surface activity, with minimum surface tension values of ≥20 mN/m, has consistently been observed in surfactant isolates from patients with ARDS (6, 10, 11) or severe pneumonia, necessitating mechanical ventilation (10). These changes have been ascribed to a variety of biochemical alterations, including changes in the phospholipid, fatty acid, and surfactant apoprotein profiles. In addition, inhibition of surfactant function by plasma proteins leaked into the alveolar space has been established as a powerful surfactant-inhibitory mechanism (10). An in vitro study (28) documented the surfactant-inhibitory capacity to be fibrinogen > hemoglobin > albumin. Moreover, the generation of a fibrin clot from fibrinogen in the presence of pulmonary surfactant was shown to cause the most severe surfactant inhibition in another in vitro study (27). Compared with (soluble)
fibrinogen, the dose-inhibition curve of fibrin is shifted to the left by more than two orders of magnitude. The incorporation of hydrophilic surfactant compounds into the nascent fibrin matrix, with a concomitant severe loss of surface tension-lowering properties, has been suggested as the underlying mechanism. Interestingly, in vitro proteolysis of surfactant-incorporating fibrin clots was shown to be suitable for “rescue” of the surfactant material trapped in the fibrin matrix (8), with its surface tension-lowering properties being conserved.

The present study employed aerosol techniques for sequential alveolar delivery of fibrinogen and thrombin in intact rabbit lungs to create a model of selective alveolar fibrin formation. Marked deterioration of ventilation-perfusion (VA/Q) matching with a predominance of shunt flow, reminiscent of gas exchange disturbances in ARDS, occurred under these conditions, with changes far surpassing those of sole fibrinogen challenge. Rescue inhalation of either urokinase or surfactant, undertaken after formation of the alveolar fibrin clots, reduced the gas exchange abnormalities, but by far the most effective approach for restoration of gas exchange properties was the combined rescue administration of surfactant and urokinase. Targeting alveolar fibrin formation by transbronchial administration of surfactant plus urokinase might thus offer a new therapeutic strategy in acute inflammatory lung diseases with extensive hyaline membrane formation, thereby further expanding current strategies of transbronchial surfactant administration in patients with severe ARDS (7, 32).

METHODS

Materials. The mouse monoclonal anti-human D-dimer antibody (murine IgG3 κ clone DD-3B6/22) was purchased from American Diagnostic (Greenwich, CT). The antibody recognizes D-dimer and cross-linked fibrin degradation products. Bovine serum albumin (BSA-C) and silver-enhancer solution were purchased from Zellerfeld, Germany). Bovine thrombin was aerosolized with another ultrasonic nebulizer (Portasonic II, DeVilbiss Medizinische Produkte, Langen, Germany). This device produces an aerosol with a mass median aerodynamic diameter of 4.5 μm and a geometric standard deviation of 2.1 as measured with a laser diffractometer (HELOS, Sympatec, Clausthal-Zellerfeld, Germany). Bovine thrombin was aerosolized with 5% CO2 was used for ventilation (volume-controlled, positive end-expiratory pressure 1 cmH2O). The isolated lungs were then ventilated and perfused for a steady-state period lasting 30 min. Lungs that displayed visible signs of infection, gained >2 g of weight during the steady state, or showed an increase in pulmonary arterial pressure (Ppa) were excluded from the further course of the study.

Gravimetric estimation of the capillary filtration coefficient. The capillary filtration coefficient (Kfc) was determined gravimetrically, employing a sudden venous pressure elevation of 10 cmH2O for 8 min. Kfc was calculated by time (t) 0 extrapolation of the slope of the weight gain with a semilogarithmic plot of the rate of weight gain of the lung according to Taylor and Gaar (30). Vascular compliance was determined as the change in vascular volume per change in microvascular pressure. The initial rapid change in weight over the first 2 min was used for the calculation of vascular compliance (30). Retention weight was defined as the difference in weight gain before and after venous pressure elevation.

Aerosol procedures. Aerosolization of isotonic saline, human uPA, human fibrinogen (15 mg/ml), and a calf lung surfactant extract (15 mg/ml) was performed by means of an ultrasonic nebulizer (Pulmo Sonic 5500, DeVilbiss Medizinische Produkte, Langen, Germany). This device produces an aerosol with a mass median aerodynamic diameter of 4.5 μm and a geometric standard deviation of 2.1 as measured with a laser diffractometer (HELOS, Sympatec, Clausthal-Zellerfeld, Germany). Bovine thrombin was aerosolized with another ultrasonic nebulizer (Portasonic II, DeVilbiss Medizinische Produkte), with a mass median aerodynamic diameter of 4.3 μm and a geometric standard deviation of 2.2. The nebulizers were connected to the inspiratory tubing of the ventilation system. Condensation of aerosolized material within the inspiratory limb of the ventilation system was reduced by heating to 40°C. Under these conditions, a lung deposition rate of 25% of the initially generated aerosol mass was achieved (25). This deposition fraction, which was controlled periodically, was taken into consideration when calculating the quantity of agents delivered to the bronchoalveolar space by aerosolization. The total nebulized dose was obtained by weighing the nebulizer before and after the aerosolization maneuvers. In a preceding study (25), ultrasonic nebulization was ascertained not to impair the biophysical and biochemical properties of surfactant material. Similarly, the clotting ability of the fibrinogen preparation used in the present study was found to be unaffected by the aerosolization procedure (>95% clot formation both pre- and postnebulization). In contrast, the specific activities of uPA and thrombin were found to be reduced to 89 and 73%, respectively, of the initially provided activity (measured in vitro by aerosol trapping and subsequent measurement of activity by means of the chromogenic substrates S-2444 and S-2238). All data concerning the total deposited pulmonary activity of thrombin and urokinase were corrected for this loss of activity during the nebulization procedure.

VA/Q determination in isolated lungs by multiple inert gas elimination technique. The continuous VA/Q distributions were determined by the multiple inert gas elimination technique (MIGET) as described by Wagner and West (31). Determination of VA/Q distribution was performed by least squares analysis. An indication of the acceptable quality of the VA/Q distributions is a residual sum of squares of 5.348 or less in half of the experimental runs (50th percentile) or 10.645 or less in 90% of the experimental runs (90th percentile) (31). In the present study, 72.4% of the residual sum of squares were <5.348 and 98.4% were <10.645.

Experimental protocol. Seven experimental groups were employed. The treatment protocol of six groups is shown in Fig. 1. At the end of the steady-state period, a baseline VA/Q measurement was performed and time was set at zero. Nebulization of the different agents was then undertaken from t = 0 until t = 60 min, from t = 65 until t = 75 min, and from t = 95 until t = 155 min. Additional MIGET analysis was performed at 90, 165, and 195 min. The control group re-
ceived nebulized saline to ensure equivalent fluid load of the lungs. In the fibrinogen (Fbg) group, aerosolization of fibrinogen was combined with two saline nebulization periods, resulting in a total fluid loading of 7.4 g as measured for this group. In the fibrin (Fbg-Thr) group, fibrinogen was aerosolized with subsequent nebulization of thrombin to induce fibrin formation. Total lung fluid deposition was 8.6 g. Rescue applications were undertaken after the preceding fibrin formation, with the timing and dose of these rescue applications shown in Fig. 1. The protocols included the administration of surfactant (Fbg-Thr-Surf group), uPA (Fbg-Thr-uPA group), and a combination of these agents (Fbg-Thr-Surf-uPA group). The total lung fluid load measured in these experiments was 7.0, 7.4, and 7.3 g, respectively.

In a separate set of experiments, vascular permeability and gas exchange in response to high doses of inhaled urokinase-type plasminogen activator; V˙A/Q˙, ventilation-perfusion. Boxes, times of the different interventions. Arrows, times of determination of V˙A/Q˙ distribution with multiple inert gas elimination technique. Net fluid deposition, total amount of fluid volume deposited by the nebulization maneuvers.

**Fig. 1.** Overview of experimental protocols. Fbg, fibrinogen; Thr, thrombin; Surf, surfactant; uPA, urokinase-type plasminogen activator; V˙A/Q˙, ventilation-perfusion. Boxes, times of the different interventions. Arrows, times of determination of V˙A/Q˙ distribution with multiple inert gas elimination technique. Net fluid deposition, total amount of fluid volume deposited by the nebulization maneuvers.
and a mean $K_{f_p}$ value of $5.2 \times 10^{-5} \pm 1.2 \times 10^{-5}$ ml·s$^{-1}$·cmH$_2$O$^{-1}$·g lung wet weight$^{-1}$ at the end of the steady-state period. Baseline $V_{A}/Q$ measurements revealed a unimodal, narrow distribution of perfusion and ventilation to midrange $V_{A}/Q$ (0.1 < $V_{A}/Q$ < 10) areas throughout. Shunt flow ($V_{A}/Q$ < 0.005) and perfusate flow to poorly ventilated areas (low $V_{A}/Q$ areas; 0.005 < $V_{A}/Q$ < 0.1) were extremely low (< 2.5%), and no perfusion of high $V_{A}/Q$ regions (10 < $V_{A}/Q$ < 100) was observed (data not shown in detail). Dead space ventilation ($V_{A}/Q$ > 100) approximated 50% of ventilation in this system of isolated lung ventilation. These data correspond to previous investigations in the perfused rabbit lung (29) and document a physiological $V_{A}/Q$ distribution and an intact endothelial and/or epithelial barrier in these isolated organs.

Nebulization of isotonic saline (control), which was performed to mimic the total fluid burden and to characterize the effect exerted by this volume load per se, did not affect Ppa, ventilation pressure (Table 1), or $K_{f_p}$ values (data not shown in detail). A total weight gain of 7.9 g was monitored, being equivalent to the total deposited aerosol mass (8.3 g). After 155 min, there was a small but nonsignificant increase in perfusate flow to low $V_{A}/Q$ areas (maximum 2.3%; see Table 1) and in shunt flow (maximum 3.8%) as well as a concomitant very minor decrease in the perfusion of areas with normal $V_{A}/Q$ ratios.

### Table 1. Pulmonary arterial pressure, ventilation pressure, lung weight gain, perfusion of lung areas with low $V_{A}/Q_{\text{s}}$ ratios, dead space ventilation, and dispersion of perfusion

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Control</th>
<th>Fbg</th>
<th>Fbg-Thr</th>
<th>Fbg-Thr-Surf</th>
<th>Fbg-Thr-uPA</th>
<th>Fbg-Thr-Surf-uPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7.7 ± 0.7</td>
<td>7.3 ± 0.6</td>
<td>7.0 ± 0.4</td>
<td>6.9 ± 0.3</td>
<td>7.7 ± 0.2</td>
<td>7.8 ± 0.5</td>
</tr>
<tr>
<td>90</td>
<td>7.4 ± 0.8</td>
<td>7.2 ± 0.6</td>
<td>7.3 ± 0.5</td>
<td>7.1 ± 0.2</td>
<td>8.0 ± 0.4</td>
<td>8.5 ± 0.5</td>
</tr>
<tr>
<td>195</td>
<td>7.6 ± 0.8</td>
<td>7.6 ± 0.6</td>
<td>8.7 ± 0.7</td>
<td>8.1 ± 0.4</td>
<td>9.7 ± 0.8</td>
<td>9.9 ± 0.9</td>
</tr>
</tbody>
</table>

**Pulmonary arterial pressure, mmHg**

<table>
<thead>
<tr>
<th>Ventilation pressure, mmHg</th>
<th>0</th>
<th>8.3 ± 0.4</th>
<th>8.1 ± 0.2</th>
<th>8.5 ± 0.2</th>
<th>8.6 ± 0.3</th>
<th>8.9 ± 0.2</th>
<th>8.3 ± 0.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>90</td>
<td>8.2 ± 0.3</td>
<td>9.6 ± 0.1*</td>
<td>9.4 ± 0.2*</td>
<td>9.9 ± 0.2*</td>
<td>9.6 ± 0.2*</td>
<td>10.4 ± 0.1†</td>
<td></td>
</tr>
<tr>
<td>195</td>
<td>8.4 ± 0.4</td>
<td>9.7 ± 0.3*</td>
<td>10.7 ± 0.2*</td>
<td>10.7 ± 0.2†</td>
<td>10.2 ± 0.7*</td>
<td>10.8 ± 0.4*</td>
<td></td>
</tr>
</tbody>
</table>

**Lung weight gain, g**

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>0 ± 0</th>
<th>0 ± 0</th>
<th>0 ± 0</th>
<th>0 ± 0</th>
<th>0 ± 0</th>
<th>0 ± 0</th>
</tr>
</thead>
<tbody>
<tr>
<td>90</td>
<td>5.9 ± 0.8</td>
<td>7.9 ± 1.3</td>
<td>7.5 ± 0.6</td>
<td>10.4 ± 1.1</td>
<td>8.4 ± 0.5</td>
<td>9.0 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>195</td>
<td>7.9 ± 0.7</td>
<td>10.7 ± 1.8</td>
<td>12.5 ± 1.3*</td>
<td>18.2 ± 1.8*</td>
<td>18.9 ± 4.8*</td>
<td>14.0 ± 1.5*</td>
<td></td>
</tr>
</tbody>
</table>

**Low $V_A/Q$, %Q**

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>0.1 ± 0.1</th>
<th>0.1 ± 0.1</th>
<th>0.1 ± 0.1</th>
<th>0.0 ± 0.0</th>
<th>0.6 ± 0.5</th>
<th>0.2 ± 0.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>90</td>
<td>1.9 ± 0.6</td>
<td>2.6 ± 1.5</td>
<td>1.1 ± 1.0</td>
<td>0.1 ± 0.1</td>
<td>0.7 ± 0.5</td>
<td>1.1 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>195</td>
<td>2.3 ± 0.9</td>
<td>1.3 ± 1.1</td>
<td>4.5 ± 1.4</td>
<td>5.4 ± 3.2</td>
<td>3.6 ± 1.9</td>
<td>2.4 ± 1.7</td>
<td></td>
</tr>
</tbody>
</table>

**Log $SD_Q$**

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>0.41 ± 0.08</th>
<th>0.42 ± 0.07</th>
<th>0.43 ± 0.05</th>
<th>0.36 ± 0.04</th>
<th>0.42 ± 0.09</th>
<th>0.36 ± 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>90</td>
<td>0.56 ± 0.12</td>
<td>0.82 ± 0.23</td>
<td>1.21 ± 0.30*</td>
<td>0.51 ± 0.07</td>
<td>0.59 ± 0.09</td>
<td>0.46 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>195</td>
<td>0.68 ± 0.13</td>
<td>0.69 ± 0.19</td>
<td>1.25 ± 0.18*</td>
<td>0.75 ± 0.20</td>
<td>0.83 ± 0.11</td>
<td>0.91 ± 0.20</td>
<td></td>
</tr>
</tbody>
</table>

**Dead space ventilation, %V**

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>45.6 ± 2.1</th>
<th>47.8 ± 2.6</th>
<th>44.9 ± 2.6</th>
<th>53.7 ± 1.3</th>
<th>49.9 ± 5.3</th>
<th>45.4 ± 2.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>90</td>
<td>55.3 ± 2.2</td>
<td>54.0 ± 3.3</td>
<td>57.6 ± 1.9</td>
<td>51.6 ± 2.4</td>
<td>54.0 ± 2.2</td>
<td>51.3 ± 1.9</td>
<td></td>
</tr>
<tr>
<td>195</td>
<td>56.4 ± 3.4</td>
<td>52.6 ± 2.1</td>
<td>60.2 ± 1.6</td>
<td>63.4 ± 4.0</td>
<td>59.9 ± 3.3</td>
<td>57.8 ± 1.0</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. $V_{A}/Q$, ventilation-perfusion; Fbg, fibrinogen; Thr, thrombin; Surf, surfactant; uPA, urokinase-type plasminogen activator; low $V_{A}/Q$, perfusion of areas with 0.005 < $V_{A}/Q$ < 0.1; log $SD_Q$, log SD of perfusion; dead space ventilation, ventilation of areas with $V_{A}/Q > 100$. Significant difference from control: *$P < 0.05$; †$P < 0.01$.

Vascular permeability and gas exchange in response to inhaled uPA and thrombin. Nebulization of 9.4 U/kg body wt of thrombin did not affect Ppa, weight gain, ventilation pressure (Table 2), or lung gas exchange (data not shown in detail). $K_{f_p}$, vascular compliance, and retention (weight gain during venous pressure challenge) remained unchanged (Table 2). Similarly, lung deposition of 4,111 ± 564 U uPA/kg body wt did not alter $K_{f_p}$, vascular compliance, retention, and Ppa (Table 2). However, when uPA doses of 6,106 ± 889 and 10,227 ± 2,031 U/kg body wt were provided to the alveolar compartment, significant increases in $K_{f_p}$ values up to 11.9 ± 10.5 ± 3.3 ± 10.5 and 22.2 ± 2.5 ± 2.9 ± 10.5 ml·s$^{-1}$·cmH$_2$O$^{-1}$·g lung wet weight$^{-1}$, respectively, were noted (Table 2). The highest uPA dose also caused a marked increase in fluid retention (weight gain) during the venous challenge maneuver. Ppa, vascular compliance, and ventilation pressure remained unchanged.

**Effects of fibrinogen deposition and fibrin formation in the alveolar compartment.** Aerosolization of fibrinogen resulted in a moderate increase in shunt flow from 1.2 ± 0.3 to 7.4 ± 2.1% and a slight increase in perfusion to poorly ventilated areas to 1.3 ± 1.1% (Table 1). Dead space ventilation increased by ~5%. Ppa remained constant (7.2 ± 0.4 mmHg at 0 min and 7.4 ± 0.4 mmHg at 195 min), whereas lung weight displayed a progressive increase, resulting in a total...
bution in the midrange V˙A/Q˙ areas occurred, indicated
ventilation, and a broadening of the perfusion distri-

6
1.4%; Table 1). Dead space ventilation increased sig-

vealed a homogeneous distribution of staining inten-

Distribution among the different regions of the lung
ways was noted, however, without airway occlusion.

increase in ventilation pressure was noted (Table 1).

increase to 12.5 \pm 1.3 g (8.6 g of deposited aerosol) at

weight gain of 10.7 \pm 1.8 g at the end of the experi-

weight gain induced by the maneuver of venous pressure
\text{elevation for determination of } K_{fe}. \text{ Thrombin, 9.4 } \text{U/kg body wt; uPA dose I, 4.111 \pm 564 \text{ U/kg body wt; uPA dose II, 6.106 \pm 889 \text{ U/kg body wt; uPA dose III, 10.227 \pm 2.031 \text{ U/kg body wt. Significant difference from time 0 measurement: } *P < 0.05; †P < 0.01.}

weight gain of 10.7 \pm 1.8 g at the end of the experi-
ments. As in the control group, the major part of this
weight gain was, however, due to the total deposited
aerosol mass of 7.4 g.

Induction of fibrin formation by sequential aerosol
application of fibrinogen and thrombin caused severe
deterioration of V˙A/Q˙ matching, with a progressive
increase in shunt flow to 22.4 \pm 1.4% (Fig. 2) and some
additional perfusion of poorly ventilated areas (4.5 \pm 
1.4%; Table 1). Dead space ventilation increased sig-
ificantly from 44.9 \pm 2.6 to 60.2 \pm 1.6% of total
ventilation, and a broadening of the perfusion distri-
bution in the midrange V˙A/Q˙ areas occurred, indicated
by an increased log SD of perfusion (log SDQ˙; Table 1).
Ppa remained unaltered, whereas weight gain in-
creased to 12.5 \pm 1.3 g (8.6 g of deposited aerosol) at
the end of the experiments. In addition, some moderate
increase in ventilation pressure was noted (Table 1).

Fibrin was detected by immunohistochemistry and
was found predominantly in the alveolar compartment
(Fig. 4). Some minor deposition in the conducting air-
ways was noted, however, without airway occlusion.
Distribution among the different regions of the lung
was rather homogeneous, and image analysis also re-
vealed a homogeneous distribution of staining intensi-

Rescue administration of surfactant and/or uPA. In
lungs with preceding fibrin formation due to the com-
bined application of fibrinogen and thrombin, aerosol
application of surfactant resulted in a significant de-
crease in shunt flow to 12.1 \pm 2.7%. The percentage of
perfusion of low V˙A/Q˙ areas was largely unchanged
by this intervention (5.4 \pm 3.2%; Table 1). Perfusion of
normal V˙A/Q˙ areas was thus increased compared with
that in the Fbg-Thr group (Fig. 3). Dead space ventila-
tion was 63.4% at the end of the experiments. V˙A/Q
mismatch, indicated by log SDQ˙, was reduced com-
pared with that in the Fbg-Thr group (Table 1). Ppa
was not altered by this intervention. Total lung weight

gain was 18.2 \pm 1.8 g at the end of the experiments (7
g of deposited fluid volume) in the Fbg-Thr-Surf group,
and some increase in ventilation pressure occurred.

Aerosol application of 4,588 U/kg body wt of uPA
subsequent to alveolar fibrin formation resulted in a
moderate reduction in pulmonary shunt flow (17.5% at
the end of the experiments), which was not signifi-
cantly different from that in the Fbg-Thr group (Fig. 2).
Perfusion of low V˙A/Q˙ areas was reduced (3.6%; Table
1), and that of normal V˙A/Q˙ areas was increased (Fig.
3). The broadening of perfusion distribution in midrange
V˙A/Q˙ areas (log SDQ˙) was not different from
that in the Fbg-Thr group (Table 1). At the end of the
experiments, dead space ventilation was 60% and Ppa
was 9.7 mmHg. Total weight gain at this time was
18.9 g (7.4 g of deposited fluid), and peak ventilation
pressure increased to a mean of 10.2 mmHg.

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Thrombin</th>
<th>Dose I</th>
<th>Dose II</th>
<th>Dose III</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.1 ± 0.4</td>
<td>5.4 ± 0.6</td>
<td>5.1 ± 0.4</td>
<td>4.7 ± 1.2</td>
</tr>
<tr>
<td>70</td>
<td>6.3 ± 1.1</td>
<td>6.3 ± 1.4</td>
<td>6.4 ± 1.5</td>
<td>13.2 ± 2.3*</td>
</tr>
<tr>
<td>120</td>
<td>6.5 ± 0.9</td>
<td>5.9 ± 1.6</td>
<td>11.9 ± 3.6*</td>
<td>22.2 ± 4.6†</td>
</tr>
</tbody>
</table>

Vascular compliance, ml/cmH2O

<table>
<thead>
<tr>
<th>Retention, g</th>
<th>0</th>
<th>1.6 ± 0.4</th>
<th>1.6 ± 0.5</th>
<th>1.7 ± 0.5</th>
<th>1.0 ± 0.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>70</td>
<td>1.2 ± 0.3</td>
<td>1.5 ± 0.1</td>
<td>2.0 ± 0.1</td>
<td>4.3 ± 1.1*</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>1.8 ± 0.3</td>
<td>2.0 ± 0.2</td>
<td>2.6 ± 0.2*</td>
<td>7.8 ± 3.0†</td>
<td></td>
</tr>
</tbody>
</table>

Pulmonary arterial pressure, mmHg

| Ventilation pressure, mmHg | 0 | 9.8 ± 1.1 | 9.5 ± 1.4 | 8.8 ± 1.4 | 8.5 ± 1.3 |
|                           | 70| 8.3 ± 1.0 | 9.2 ± 1.1 | 9.3 ± 1.1 | 9.8 ± 1.0 |
|                           | 120| 9.0 ± 1.2 | 9.1 ± 1.6 | 9.2 ± 1.6 | 11.2 ± 1.9 |

Values are means ± SE. K_{fe}, capillary filtration coefficient; retention, weight gain induced by the maneuver of venous pressure elevation for determination of K_{fe}. Thrombin, 9.4 U/kg body wt; uPA dose I, 4.111 ± 564 U/kg body wt; uPA dose II, 6.106 ± 889 U/kg body wt; uPA dose III, 10.227 ± 2.031 U/kg body wt. Significant difference from time 0 measurement: *P < 0.05; †P < 0.01.

Fig. 2. Intrapulmonary shunt flow in response to different experi-
mental protocols. Shunt flow (V˙A/Q < 0.005) is given in percent of
total lung perfusion (Q). Horizontal bars, times of the different
maneuvers. Values are means ± SE of 6 independent experiments/
group; error bars not seen are within symbol. Significant difference
compared with Fbg-Thr lungs without rescue application of uPA
and/or surfactant: *P < 0.05; **P < 0.01.
Combined aerosol application of uPA and surfactant resulted in a highly significant decrease in intrapulmonary shunt flow (Fig. 2) to 6.8% at the end of the experiments. Notably, this regimen was the only one that not only inhibited the further increase in shunt flow but also reversed the extent of shunt flow, which already represented ~12% after termination of alveolar fibrin formation in this group. Similarly, perfusion of low V\text{A}/Q\text{\acute{a}} areas was reduced to 2.4% at the end of the experiments (Table 1). As a result, perfusion of normal V\text{A}/Q\text{\acute{a}} areas was markedly increased and approached the data with fibrinogen application alone (Fig. 3). Dead space ventilation was 57.8% in this group at the end of the study, and total weight gain was 14.0 g, with half of this weight gain (7.3 g) resulting from the nebulization maneuvers per se. As detailed in Table 1, Ppa and ventilation pressure were not substantially influenced by the rescue administration of surfactant and uPA.

**BAL fluid analysis.** Measurement of the BAL fluid D-dimer levels showed very low concentrations of this scission product of cross-linked fibrin in the control and fibrinogen-treated lungs (Fig. 5). Subsequent to alveolar fibrin formation due to additional thrombin aerosolization (Fbg-Thr group), the BAL fluid D-dimer content increased significantly, and this also held true for the surfactant-treated (Fbg-Thr-Surf) group. The highest D-dimer levels in the BAL fluid were encountered in the two uPA treatment (Fbg-Thr-uPA and Fbg-Thr-Surf-uPA) groups.

**DISCUSSION**

The present study was performed in a model of buffer-perfused, ventilated rabbit lungs previously characterized in detail (29). The advantages of this model are 1) control of pulmonary hemodynamics, continuous monitoring of lung weight, and repetitive assessment of the K_{fc}, 2) the absence of bloodborne components that might possibly confound the effects caused by selective alveolar fibrin formation, and 3) the suitability to employ aerosol technology for alveolar drug delivery. In addition, MIGET, previously adapted to this isolated lung model (33), offers a powerful tool to analyze gas exchange conditions in detail (23). As documented by baseline measurements, hemodynamics, endothelial

---

Fig. 3. Perfusion of lung areas with normal V\text{A}/Q\text{\acute{a}} ratios in response to different experimental protocols. Flow to normal V\text{A}/Q\text{\acute{a}} units is given in percent of total lung perfusion. Horizontal bars, times of the different maneuvers. Values are means ± SE of 6 independent experiments/group; error bars not seen are within symbol. Significant difference compared with Fbg-Thr lungs without rescue application of uPA and/or surfactant: *P < 0.05; **P < 0.01.

Combined aerosol application of uPA and surfactant resulted in a highly significant decrease in intrapulmonary shunt flow (Fig. 2) to 6.8% at the end of the experiments. Notably, this regimen was the only one that not only inhibited the further increase in shunt flow but also reversed the extent of shunt flow, which already represented ~12% after termination of alveolar fibrin formation in this group. Similarly, perfusion of low V\text{A}/Q\text{\acute{a}} areas was reduced to 2.4% at the end of the experiments (Table 1). As a result, perfusion of normal V\text{A}/Q\text{\acute{a}} areas was markedly increased and approached the data with fibrinogen application alone (Fig. 3). Dead space ventilation was 57.8% in this group at the end of the study, and total weight gain was 14.0 g, with half of this weight gain (7.3 g) resulting from the nebulization maneuvers per se. As detailed in Table 1, Ppa and ventilation pressure were not substantially influenced by the rescue administration of surfactant and uPA.

**BAL fluid analysis.** Measurement of the BAL fluid D-dimer levels showed very low concentrations of this scission product of cross-linked fibrin in the control and fibrinogen-treated lungs (Fig. 5). Subsequent to alveolar fibrin formation due to additional thrombin aerosolization (Fbg-Thr group), the BAL fluid D-dimer content increased significantly, and this also held true for the surfactant-treated (Fbg-Thr-Surf) group. The highest D-dimer levels in the BAL fluid were encountered in the two uPA treatment (Fbg-Thr-uPA and Fbg-Thr-Surf-uPA) groups.

**DISCUSSION**

The present study was performed in a model of buffer-perfused, ventilated rabbit lungs previously characterized in detail (29). The advantages of this model are 1) control of pulmonary hemodynamics, continuous monitoring of lung weight, and repetitive assessment of the $K_{fc}$, 2) the absence of bloodborne components that might possibly confound the effects caused by selective alveolar fibrin formation, and 3) the suitability to employ aerosol technology for alveolar drug delivery. In addition, MIGET, previously adapted to this isolated lung model (33), offers a powerful tool to analyze gas exchange conditions in detail (23). As documented by baseline measurements, hemodynamics, endothelial

---

Fig. 4. Immunogold-silver staining and nuclear fast red counterstaining of fibrin in rabbit lung tissue. A: bright-field image. Fibrin is detected within the alveolar spaces. B: epipolarization depiction of immunogold-silver-stained fibrin. C: pseudocolor conversion of the epipolarization image allows visualization of the staining intensity. Arrows, alveolar septa.
and/or epithelial barrier function, and \( V_{A}/Q \) matching are well within the physiological range in these buffer-perfused lungs.

In the control experiments, nebulization of saline was undertaken to mimic the extent of alveolar fluid deposition effected by the experimental protocols employing fibrinogen aerosolization. Interestingly, the net lung fluid loading of \( \approx \)8 g, assessed both by measurement of aerosolized mass and by monitoring of lung weight gain, did not affect pulmonary hemodynamics, vascular integrity, and ventilation pressure at all. Moreover, physiological \( V_{A}/Q \) matching was virtually fully maintained, with only minimal shunt flow (\( \approx \)3%) noted at the end of the experiments. Thus alveolar fluid deposition per se, resulting in an approximate doubling of the baseline lung weight, is apparently well tolerated with respect to gas exchange in these intact lungs.

Alveolar deposition of a fibrinogen solution, again causing an \( \approx \)8-g net lung weight, provoked an increase in shunt flow to \( \approx \)7% along with a very minor increase in perfusion of low \( V_{A}/Q \) areas and a corresponding decrease in perfusion of normal \( V_{A}/Q \) regions. This finding is very compatible with an in vitro study (28) that demonstrated the surfactant-inhibitory capacities of fibrinogen. When the ratio of aerosolized fibrinogen (\( \approx \)20 mg/kg body wt) to the endogenous rabbit alveolar surfactant pool (\( \approx \)7 mg/kg (25)) is calculated, some moderate inhibition of surfactant function is to be anticipated from the in vitro studies, and this might well cause some limited extent of atelectasis and shunt flow.

In vitro studies addressing the surfactant-inhibitory capacity of proteins also forwarded the concept that hydrophobic surfactant components, phospholipids, and the surfactant apoproteins B and C are incorporated into a nascent fibrin matrix and that nearly complete incorporation of these surfactant constituents, with severe inhibition of surface activity, already occurs at a fibrin-to-surfactant ratio of 1:5 (27). This study is the first one to rigorously test this concept in intact lungs by sequential aerosolization of fibrinogen and thrombin, the latter being applied at a dose that per se does not induce pulmonary abnormalities. With this approach, the generation of up to \( \approx \)20 mg/kg body wt of fibrin was to be anticipated, and the appearance of alveolar fibrin deposits, very much resembling hyaline membrane formation, was nicely demonstrated by the immunohistological studies. Indeed, a marked progressive shunt flow, surpassing 20% at the end of the experiments, as well as perfusion of low \( V_{A}/Q \) areas (4–5%) was noted in these lungs, indicating severe surfactant deterioration, with a loss of alveolar patency in the fibrin-loaded lungs. Concomitantly, the perfusion of normal \( V_{A}/Q \) areas was significantly decreased. Thus fibrin generation in the alveolar compartment is apparently a potent surfactant-inhibitory mechanism, with a severe impact on gas exchange under conditions of intact lung architecture. This finding also supports previous investigations (22, 24, 25) employing different surfactant-inhibitory interventions in intact lungs (e.g., lavage, detergents, acid aspiration) in which a deterioration of \( V_{A}/Q \) matching with a predominance of shunt flow was consistently demonstrated.

In several of these previously reported models of surfactant inhibition (18–20, 24, 25), transbronchial administration of exogenous surfactant either by instillation or by aerosolization was found to improve gas exchange and, in particular, to reduce shunt flow, compatible with restoration of alveolar surfactant function. Even when applied as an aerosol, surfactant might reach atelectatic lung regions due to its adsorption and lateral spreading facilities. It is in line with these observations that a significant improvement in gas exchange was noted in response to surfactant nebulization, undertaken subsequent to the current protocol of alveolar fibrin formation. The presently deposited surfactant quantity of \( \approx \)15 mg/kg body wt is well in line with previous experimental surfactant replacement strategies employing aerosol technology (12, 18, 20, 24, 25). However, the overall efficacy of the transbronchial surfactant administration was somewhat limited in the present study in fibrin-loaded lungs compared with that in lavage models (18, 20, 24). The most reasonable explanation for this finding is the assumption that the presence of fibrin interferes with the entrance of surfactant into the alveolar spaces or that even strong apposition of alveolar walls (“fibrin gluing”) may occur.

To target alveolar fibrin formation more directly, nebulization of urokinase was undertaken as a rescue regimen. When this approach was tested in preceding pilot experiments, the pulmonary deposition of \( \approx \)10,000 U/kg body wt of uPA was found to significantly increase the \( K_{p} \), which may be due to the lysis of extracellular matrix components, with subsequent enhanced fluid flux under conditions of an increased pulmonary venous pressure challenge. In contrast, pul-
monary deposition of about 4,500 U/kg body wt of urokinase did not exert any effect on lung barrier properties, ventilation pressure, and pulmonary hemodynamics. This dose appears to be low compared with the uPA doses currently used in acute myocardial infarction, where 40,000–50,000 U/kg body wt may be employed (26). It has to be kept in mind, however, that the volume of the bronchoalveolar lining layer, to which the nebulized urokinase is first distributed, is lower by many orders of magnitude than the circulating blood volume, even under conditions of lung edema formation. Moreover, measurement of the perfusate levels of urokinase did not detect rapid passage of the aerosolized protease into the intravascular space (ELISA technique; data not shown). The efficacy of nebulized urokinase to cause fibrin lysis in the alveolar space is documented by the increase in lavage fluid D-dimer levels in response to this regimen. It is known from an in vitro study (8) that a urokinase attack on surfactant-incorporating fibrin is capable of liberating functionally intact surfactant, which might then be assumed to improve surface tension properties at the alveolar surface. Indeed, some retardation of shunt flow development and perfusion of low V\textsubscript{A}/Q\textsubscript{A} areas was noted in the urokinase-treated lungs; however, the data did not significantly differ from the values in nontreated controls. Thus, although showing some efficacy, the presently employed dose of urokinase, designed to avoid induction of lung permeability increase by this protease, resulted only in a minor improvement of gas exchange in the fibrin-loaded lungs.

Most interestingly, the combined aerosol administration of urokinase and surfactant turned out to be the most effective approach for improvement of V\textsubscript{A}/Q\textsubscript{A} matching in the present model of alveolar fibrin deposition. Notably, not only retardation of shunt development but reversal of the extent of shunt flow was achieved by this approach, with percentages of shunt at the end of the experiments being in the same range as in the lungs undergoing fibrinogen-alone administration (≈7%). In addition, low numbers of low V\textsubscript{A}/Q\textsubscript{A} areas were noted, thus resulting in a markedly increased flow to lung units with normal V\textsubscript{A}/Q\textsubscript{A} ratios. Thus one very plausible explanation might be that the surfactant facilitated the distribution of urokinase to the fibrin-loaded alveolar spaces (surfactant as carrier), thereby enhancing the overall fibrinolytic efficacy of the nebulized uPA and converting most fibrin into fibrin split products; the surfactant-inhibitory capacity of the split products only slightly surpasses that of fibrinogen. However, lavage fluid D-dimer levels were not found to be increased in the urokinase-surfactant group compared with the urokinase-alone group. Thus, as an alternative explanation, some limited urokinase-induced fibrinolysis might allow better access of the exogenously administered surfactant material to the alveolar compartment, thereby enhancing the beneficial impact of the exogenous surfactant material on surface tension regulation and alveolar stability.

In conclusion, the present study demonstrates that alveolar fibrin formation, induced in an intact lung with otherwise physiological function, provokes severe gas exchange abnormalities, with a predominance of shunt flow. This finding is very compatible with the hypothesis that fibrin formation in the alveolar compartment is a potent inhibitor of surfactant function, resulting in a loss of alveolar stability. Rescue administration of surfactant and urokinase, both aerosolized after formation of the fibrin deposits, reduced the progressive development of shunt flow in this model, with surfactant being more potent. The most efficient approach, however, was the conebulization of surfactant and urokinase, which reversed shunt flow and markedly increased perfusion of normal V\textsubscript{A}/Q\textsubscript{A} areas. Clearly, it has to be kept in mind that these results were obtained in an in vitro model, not under in vivo conditions of acute respiratory failure. However, the proof of the concept (high efficacy of conebulization of surfactant and urokinase) was beyond doubt documented in this model. Thus combined surfactant and urokinase administration may indeed offer a new therapeutic regimen in conditions with marked and persisting alveolar fibrin formation such as severe or delayed ARDS and pneumonia demanding mechanical ventilation (9, 16) and rapidly progressive idiopathic lung fibrosis (3, 17), in which each agent alone might not have easy access to the fibrin-filled alveolar spaces. The currently employed dose of urokinase was chosen to avoid induction of vascular leakage, but further studies in intact animal models will be mandatory to figure out whether coaerosolization of surfactant and a fibrinolytic agent may be operative without provoking lung bleeding. Beyond the present target variable (restoration of gas exchange), this approach may also be of interest in view of the hypothesis that delayed clearance of fibrin in inflammatory lung diseases may favor the progression into lung fibrosis, with surfactant-depleted and fibrin-glued alveolar spaces serving as nidi for fibroblast invasion (concept of collapse induration) (2).

Our gratitude goes to Dr. P. D. Wagner (University of California, San Diego, La Jolla, CA) for supplying the computer program. We also thank R. L. Snipes (Department of Anatomy, Justus-Liebig-University Giessen, Giessen, Germany) for linguistically reviewing this manuscript.

This work was supported by the Deutsche Forschungsgemeinschaft Grant SFB 547.

REFERENCES
Alveolar Fibrin and Conevulization of uPA and Surfactant


