Alveolar environment influences the metabolic and biophysical properties of exogenous surfactants

Pramod S. Puligandla, Tara Gill, Lynda A. McCaig, Li-Juan Yao, Ruud A. W. Veldhuizen, Fred Possmayer, and James F. Lewis. Alveolar environment influences the metabolic and biophysical properties of exogenous surfactants. J. Appl. Physiol. 88: 1061-1071, 2000.—Several factors have been shown to influence the efficacy of exogenous surfactant therapy in the acute respiratory distress syndrome. We investigated the effects of four different alveolar environments (control, saline-lavaged, N-nitroso-N-methylurethane, and hydrochloric acid) on the metabolic and functional properties of two exogenous surfactant preparations: bovine lipid extract surfactant and recombinant surfactant-associated protein (SP) C drug product (rSPC) administered to each of these groups. The main difference between these preparations was the lack of SP-B in the rSPC. Our results demonstrated differences in the large aggregate pool sizes recovered from each of the experimental groups. We also observed differences in SP-A content, surface area cycling characteristics, and biophysical activities of these large aggregate forms after the administration of the two exogenous surfactant preparations. We conclude that the alveolar environment plays a critical role, influencing the overall efficacy of exogenous surfactant therapy. Thus further preclinical studies are warranted to investigate the specific factors within the alveolar environment that lead to the differences observed in this study.

ALTERATIONS IN THE ENDOGENOUS surfactant system have been implicated in the lung dysfunction observed in patients with the acute respiratory distress syndrome (ARDS) (9, 22). Consequently, exogenous surfactant administration has been tested as a therapeutic modality for these patients. Results of clinical trials have been inconsistent (1, 10), no doubt because several factors may influence a host's response to this therapy. Some of these factors include the timing of exogenous surfactant administration over the course of the injury, the method used to deliver the surfactant, the specific surfactant preparation utilized, and the mode of mechanical ventilation used after the surfactant has been administered (23, 30).

The optimization of all these factors in an individual patient may prove to be difficult. For example, when an exogenous surfactant preparation is selected for patients with ARDS, a product with superior in vitro biophysical properties would conventionally be used. Several preterm animal studies have shown, however, that the efficacy of a surfactant preparation in vivo may not be reflected by these in vitro functional assessments (5, 14). This observation was supported by studies showing that an exogenous surfactant "interacted" with some components of the host's alveolar environment (16, 29). These interactions affected the function of the exogenous surfactant once it was deposited within the air space and, consequently, the efficacy of that surfactant in vivo. These observations illustrate the complexity of surfactant therapy in acute lung injury and suggest that a greater understanding of how exogenous surfactant is handled within the air space of the injured lung is required. Because the population of patients afflicted with ARDS is quite diverse, it is possible that certain types of patients with ARDS will require specific surfactant treatment strategies based on the nature of their endogenous surfactant system at the time of treatment. The purpose of this study was to characterize the metabolic and biophysical properties of two different exogenous surfactant preparations when administered to four different groups of rabbits representing distinct alveolar environments. Some of these groups were meant to reflect the different types of lung injuries observed in patients with ARDS, including direct and indirect pulmonary insults.

MATERIALS AND METHODS

Animal Preparation

Adult New Zealand White rabbits weighing 2.3 ± 0.2 kg were premedicated with intramuscular ketamine (50 mg/kg) and acepromazine (0.05 mg/kg). Animals then underwent tracheostomy and carotid artery catheterization for mechanical ventilation and arterial blood gas (ABG) measurements, respectively. Lidocaine (1%) was used for local anesthesia at all surgical sites. The arterial line provided vascular access for the intermittent infusion of saline, general anesthetic
(5–10 mg/kg of 0.8% thiopental sodium), and neuromuscular blockers (0.05 mg/kg of pancuronium bromide) and was connected to a pressure transducer for the monitoring of blood pressure and heart rate. Animals were ventilated with a pressure-limited infant ventilator (model IV-100B, Sechrist, Anaheim, CA) at a peak inspiratory pressure (PIP) necessary to maintain a tidal volume (VT) of 10 ml/kg, which was verified by a pneumotachometer (Hans Rudolph, Kansas City, MO). Other ventilatory parameters included a respiratory rate of 30 breaths/min, a fraction of inspired O₂ (F₁O₂) of 1.0, a positive end-expiratory pressure of 5 cmH₂O, and an inspira-
tory-to-expiratory ratio of 1.1. Animals were monitored for 5–10 min after the initial instrumentation to ensure animal viability and stability and to standardize physiological and ventilatory parameters as determined by the initial VT measurements and ABG analysis. Physiological monitoring (blood pressure, VT, and ABG) and handling were similar for all animals to minimize the effects of these variables on their subsequent outcome.

Animal Groups

Four different groups of animals were studied, each representing a different alveolar environment.

Saline-lavage rabbits. Once stabilized on the ventilator, a group of normal animals was disconnected from the ventilator, and warmed (37°C) 0.15 M NaCl (30 ml/kg) was instilled into the lungs through the proximal end of the endotracheal tube, as previously described (17). This was followed by gentle suctioning with a 60-ml syringe, with subsequent reconnect-
tion to the ventilator on completion of suctioning. The PIP was then adjusted to maintain a VT of 10 ml/kg verified by the pneumotachometer. This lavage procedure was repeated every 10 min until the ratio of arterial PO₂ (PaO₂) to F₁O₂ (PaO₂/F₁O₂) fell below 100 Torr. The total number of lavages was recorded, as was the time between the first and last lavage. After the final lavage, animals were ventilated for a further 30 min. This model is well established in this labora-
tory and has been used previously to assess factors that have been shown to influence the efficacy of exogenous surfactant therapy (17). These saline-lavage (lavaged) animals repre-
sented a surfactant-depleted alveolar environment.

N-nitroso-N-methylurethane-injured rabbits. Forty-eight to
72 h after injection of N-nitroso-N-methylurethane (NNMU,
15 mg/kg sc, Kings Laboratories, Blythewood, SC), animals were stabilized on the ventilator, with ABGs measured as described above. Animals with PaO₂/F₁O₂ < 175 Torr on the initial ABG analysis were included for further evaluation. These animals were then ventilated for a further 30 min with a VT of 10 ml/kg. Previous studies have shown that NNMU-induced lung injury represents an indirect insult involving changes in the endogenous surfactant system and lung physiology similar to those observed in adult patients with ARDS (20, 26).

HCl-injured rabbits. Once stabilized on the ventilator, a group of normal animals was administered a volume of 4 ml/kg of 0.2 N HCl via intratracheal instillation to induce acute lung injury. Preliminary studies demonstrated that animal viability and stability were reliably predicted if PaO₂/F₁O₂ was 50% of pretreatment values at ~ 45 min after HCl administration. This oxygenation value was therefore used as an inclusion criterion for animals that were subsequently studied in this group. This injury represented a more direct and acute form of lung injury than subcutaneously adminis-
tered NNMU (18, 27).

Control animals. Normal animals were instrumented and prepared as described above and were subsequently ventil-
ated for 30 min with a VT of 10 ml/kg.

After animals in each group were deemed suitable for further study, they were randomized to one of three experi-
mental arms of the study, which for clarity are each described separately. The first group of animals was killed immediately after stabilization for the characterization of the endogenous surfactant system. Animals randomized to the second and third groups of the study received one of two exogenous surfactant preparations and were ventilated for a further 60 min before euthanization and characterization of their surfac-
tant systems.

Exogenous Surfactant Preparations

Two exogenous surfactant preparations were utilized for these studies. Bovine lipid extract surfactant (bLES; BLES Biochemicals, London, ON, Canada) is a natural bovine lipid extract supplied as a ready-to-use suspension with a phospho-
lipid (PL) concentration of 25 mg/ml. This preparation has a PL profile similar to that of normal surfactant. Although this surfactant does not contain surfactant-associated protein (SP) A (SP-A), it does contain SP-B and SP-C. Recombinant SP-C drug product (rSPC, Byk Gulden, Konstanz, Germany) is a surfactant made from recombinant human SP-C (2%), PL (dipalmitoylphosphatidylcholine and palmitoyloleylphospha-
tidylglycerol in a 70:30 (wt/wt) ratio), and 5% palmitic acid. It is supplied as a lyophilized powder. One hundred-milligram aliquots of rSPC were dissolved in 3.6 ml of 0.15 M NaCl to give a final concentration of 25 mg PL/ml. This surfactant preparation does not contain SP-A or SP-B. A dose of 25 mg PL/kg body wt of each surfactant was used for these experiments. Although this dose was lower than that used in clinical trials evaluating the efficacy of exogenous surfactant (1, 10), the lower quantity allowed for a more accurate assessment of the metabolic characteristics of the exogenous surfactant preparations once they were deposited within the air space. The physiological efficacy of these surfactants was therefore not an outcome parameter for these studies.

Surfactant Administration

Exogenous surfactant (4 ml/kg instillation volume) was administered in small boluses during the inspiratory phase of ventilation through a side-port adaptor located at the prox-
imal end of the endotracheal tube (17). One-half of the total volume was administered with the animal on its left side, and the remainder of the preparation was administered with the animal on its right side, with an air bolus (10 ml/kg) adminis-
tered at the end of the procedure to enhance the peripheral distribution of the surfactant. Once the instillation procedure was completed, the PIP was increased by 4 cmH₂O, or until adequate ventilation was observed, and maintained at 4 cmH₂O above preinstillation levels for the first 5 min after surfactant administration. Immediately thereafter, the PIP was adjusted appropriately to confirm a VT of 10 ml/kg with the pneumotachometer. As previously described, animals that received exogenous surfactant were ventilated for 60 min after administration. ABG analysis was performed at 5, 15, 30, 45, and 60 min after surfactant administration, with VT measurements performed at similar time intervals. Blood pressure and heart rate were also monitored continuously throughout the protocol. Animals were killed with a thiopental sodium overdose and exsanguination at the end of the 1-h ventilatory period.
Sample Processing

Immediately after euthanasia, the chest cavity of each animal was opened and the lungs were lavaged as previously described (17). Briefly, the lungs were filled with ~30 ml/kg of 0.15 M NaCl until they appeared fully distended, then the saline was withdrawn and reinflused two additional times. This procedure was repeated for a total of five lavages per animal. The total volume of lung lavage was combined, and this volume was recorded.

The lung lavage was processed within 1 h of collection, as described previously (33). Briefly, aliquots of the lung lavage were centrifuged at 150 g for 10 min at 4°C to separate surfactant from cell debris (150-g pellet). The 150-g supernatant was then centrifuged for 15 min at 40,000 g to separate the surfactant into two subfractions: the heavier, large aggregate (LA) fraction (pellet) and a lighter, small aggregate (SA) fraction (supernatant). The 150-g and LA pellets were resuspended separately in small amounts of saline, and aliquots of the whole lung lavage, 150-g pellet, and LA and SA fractions were stored at −20°C until further analysis.

Surfactant Analysis

PL pool sizes. Aliquots from the whole lung lavage, 150-g pellet, and LA and SA fractions were extracted in chloroform-methanol according to the method of Bligh and Dyer (3). Each lipid extract was then dried under nitrogen. The total phospholipid (PL) pool size was measured using the modified Duck-Chong phosphorus assay (7), and calculated PL values were expressed as milligrams of PL per kilogram of body wt.

PL composition. LA fractions recovered from the control and NNMU- and HCl-injured animals that were randomized for assessment of their endogenous surfactant systems (i.e., immediate death; n = 3/group) were subsequently analyzed for PL composition, as described previously (32). This analysis could not be performed on samples from the lavage group because of the small quantity of LA available. Samples containing ~1.25 mg of PL from the other groups were extracted with chloroform-methanol according to the method of Bligh and Dyer (3). Each PL extract was then shell dried under nitrogen. The total phosphorus pool in the whole lung lavage, 150-g pellet, and LA and SA fractions was measured using the modified Duck-Chong phosphorus assay (7), and calculated PL values were expressed as milligrams of PL per kilogram of body wt.

The PL content of each tube was then determined using the method described above. The phosphorus content was then calculated. Surface tension values at 1063 min are expressed.
cycle (11, 12). Samples were cycled for 3 h. Identical, non-
cycled samples were incubated at 37°C for the same duration
as the cycled samples. The percent conversion of LA to SA was
assessed by determining the amount of SA present in the
sample 3 h after cycling relative to the total quantity of LA
plus SA recovered at this time point. A chloroform-methanol
PL extraction process and phosphorus determination, as
described above, were performed on the pellets and superna-
tants obtained after each of the cycled and noncycled samples
were centrifuged at 40,000 g.

Sample Sizes and Statistical Analysis
Previous studies conducted in our laboratory used five or
six animals per experimental group to analyze physiological
parameters and surfactant pool sizes, and these sample sizes
have been used in the present study. Values are means ± SE.
Comparisons between two experimental groups were made
using Student’s t-test. Comparisons among more than two
groups or with repetitive measures were made using a
two-way ANOVA with the Tukey post hoc test. P < 0.05 was
considered significant.

RESULTS
The baseline characteristics of animals included in
the various groups subsequently studied are shown in
Table 1. Body weights did not differ significantly among
groups. Animals from the lavaged and NNMU- and
HCl-injured groups had significantly lower PaO2 and
significantly higher arterial PCO2 and PIP than the
control group (P < 0.05).

Endogenous Surfactant Analysis
These animals did not receive exogenous surfactant
and were killed immediately after randomization to
analyze the endogenous surfactant systems of the four
groups at the time of surfactant administration. Figure
1 illustrates the total endogenous surfactant pool as
well as the LA surfactant pool size and percent LA for
each group of animals (n = 6/group). As expected, the
smallest total and LA pool sizes were observed in the
lavaged group (P < 0.02 vs. other groups). The total
surfactant pool sizes of the control and NNMU- and
HCl-injured groups were not significantly different,
although the percentage of LA recovered in the total
pools was significantly lower in the NNMU-injured
group than in the control group (P < 0.05). This finding
was similar to previous reports involving the NNMU
model of lung injury (20).

The PL composition of the recovered LA fractions is
shown in Table 2. The composition of the lavaged group
could not be assessed because insufficient quantities of
material were recovered from this group. Although LA
recovered from the control group consisted predominantly
of phosphatidylcholine (PC), the NNMU-injured group
had significantly less PC (P < 0.01) and signific-
antly greater amounts of sphingomyelin (P < 0.01)
than the controls. LA recovered from the HCl-injured
group also had significantly less PC than the control
group (P < 0.01) and significantly greater amounts of
phosphatidylglycerol than control and NNMU-injured
groups (P < 0.05). Figure 2 shows the Western blot
analysis of the representative samples of LA loaded
from each group with their densitometric calculations.
These values revealed that the HCl-injured group had
less SP-A (54%) and the NNMU-injured group had

Table 1. Baseline physiological parameters of animals
before randomization

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Lavaged</th>
<th>NNMU</th>
<th>HCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, kg</td>
<td>2.4±0.1</td>
<td>2.2±0.1</td>
<td>2.2±0.2</td>
<td>2.2±0.2</td>
</tr>
<tr>
<td>PaO2/FIO2, Torr</td>
<td>480±10</td>
<td>71.6±6*</td>
<td>157±24*</td>
<td>256±9*</td>
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<tr>
<td>Paco2, Torr</td>
<td>43.2±12</td>
<td>54.4±4.0</td>
<td>63.8±5.1</td>
<td>66.7±5.7</td>
</tr>
<tr>
<td>PIP, cmH2O</td>
<td>11.8±0.9</td>
<td>20.5±0.8*</td>
<td>26.2±0.6*</td>
<td>19.3±1.3*</td>
</tr>
</tbody>
</table>

Values are means ± SE of 18 animals in each group. NNMU, N-
nitroso-N-methylurethane-injured animals; HCl, HCl-injured ani-
mals; PaO2, arterial O2; FIO2, fraction of inspired O2; Paco2, arterial
PCO2; PIP, peak inspiratory pressure. All animals were ventilated on
an FIO2 of 1.0 for duration of study. *P < 0.05 vs. control.

Table 2. Phospholipid composition of LA recovered
from animals killed before administration
of exogenous surfactant

<table>
<thead>
<tr>
<th>Phospholipid</th>
<th>Control</th>
<th>NNMU</th>
<th>HCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphatidylcholine</td>
<td>86.9±1.2</td>
<td>58.9±2.1*</td>
<td>64.6±2.6*</td>
</tr>
<tr>
<td>Phosphatidylglycerol</td>
<td>2.1±0.2</td>
<td>2.6±1.1</td>
<td>12.5±1.8†</td>
</tr>
<tr>
<td>Sphingomyelin</td>
<td>2.0±0.9</td>
<td>22.4±0.9*</td>
<td>7.1±1.4†</td>
</tr>
<tr>
<td>Phosphatidylethandamine</td>
<td>0.9±0.05</td>
<td>1.2±0.3</td>
<td>3.8±0.5</td>
</tr>
</tbody>
</table>
| Phosphatidylinositol/phos-
| patidylserine              | 6.8±1.6 | 13.0±2.4| 10.6±1.3|
| Lysophosphatidylcholine    | 1.4±0.2 | 2.0±0.2 | 1.4±0.1|

Values are means ± SE of 3 animals in each group. LA, large
aggregate fraction. *P < 0.05 vs. control; †P < 0.05 vs. NNMU.
more SP-A (187%) for the same quantity of PL loaded. In the lavaged group, values were 98% of control (Fig. 2). Although these are representative values, patterns of recovery were similar for all samples loaded from each group (data not shown).

The total protein recovered in the lung lavage of these animals at death is shown in Table 3. More protein was obtained from the NNMU- and HCl-injured groups than from the control and lavaged groups (P < 0.001). SDS-PAGE of these lavage samples demonstrated that this protein consisted of serum proteins (data not shown). Also shown in Table 3 is the surface tension of the recovered LA fractions after 2 min of pulsation in the pulsating bubble surfactometer. LA recovered from the control and lavaged animals reduced surface tension to significantly lower values than in the NNMU- and HCl-injured groups (P < 0.05). There were no significant differences between the control and lavaged groups or between the NNMU- and HCl-injured groups.

Administration of bLES

Animals randomized to the second series of experiments received the exogenous surfactant preparation bLES and were ventilated for a further 60 min before they were killed (n = 6). Figure 3 illustrates the temporal changes in mean PaO2/FI02 over the 60 min of ventilation after the administration of bLES. PaO2/FI02 remained unchanged in the control group compared with pretreatment values, whereas an increase in PaO2/FI02 was observed in the lavaged group soon after the administration of bLES. This effect deteriorated over time. However, in the NNMU-injured group, surfactant administration resulted in a gradual but persistent increase in oxygenation over 60 min of ventilation. Finally, animals in the HCl-injured group experienced a rapid decline in PaO2/FI02 after an initial transient increase in oxygenation over the first few minutes after surfactant administration. Figure 4 illustrates the total and LA surfactant pool sizes and percent LA recovered from these animals. Total surfactant pool sizes were not significantly different among the four groups of animals at the time of death. Although the relative amounts of LA contributing to the total surfactant pool (percent LA) were lower in all three experimental groups than in the control group, this difference was statistically significant only for the NNMU- and HCl-injured groups.

Table 3. Total protein recovery and biophysical analysis of LA recovered from animals not receiving exogenous surfactant

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Control</th>
<th>Lavaged</th>
<th>NNMU</th>
<th>HCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein recovery, mg/kg</td>
<td>6</td>
<td>11±1</td>
<td>22±2</td>
<td>252±41†</td>
<td>359±31†</td>
</tr>
<tr>
<td>Surface tension, mN/m</td>
<td>3</td>
<td>5.1±0.7</td>
<td>7.8±1.8</td>
<td>17.4±3.7†</td>
<td>18.7±2.1†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of animals. *P < 0.05 vs. control; †P < 0.05 vs. lavaged.
injured groups (P < 0.05 vs. control). Representative Western blot samples utilized for densitometric analysis (similar PL quantities) of these LA (Fig. 5) showed less SP-A in the samples obtained from the lavaged group (18% of control) than in samples from the other three groups. LA isolated from the HCl-injured group (54% of control) also contained less SP-A than LA isolated from the control and NNMU-injured (169% of control) groups.

Table 4 shows the total protein recovery from the lung lavage samples at the time the animals were killed. The greatest amount of protein was recovered from the HCl-injured group (P < 0.05 vs. all groups), although protein recovery was significantly elevated in the lavaged and NNMU-injured groups compared with the control group (P < 0.05). Table 4 also shows the surface tension values of the LA recovered from these animals after 2 min of pulsation in the PBS. Interestingly, LA from the NNMU-injured animals had the lowest surface tension values of all groups of animals (P < 0.05 vs. lavage). Minimum surface tension values (R_{min}) for the lavaged and HCl-injured groups were greater than control values, although these differences did not reach statistical significance.

Results of the surface area cycling experiments performed on the LA isolated from the animals receiving exogenous bLES are shown in Fig. 6. The in vitro conversion of these LA to SA was assessed by determining the quantity of SA in each sample after 3 h of cycling at 37°C. The percentage of SA present after cycling native bLES was 73 ± 4%. There was significantly less conversion of LA to SA in the NNMU- and HCl-injured groups than in the control group (P < 0.05) and than in the lavaged group (P < 0.05) and native bLES (P < 0.01). Less than 7% SA was recovered from all samples incubated at 37°C that did not undergo surface area cycling.

Administration of rSPC

A similar series of experiments were conducted on separate groups of animals receiving rSPC rather than bLES. Figure 7 illustrates the temporal changes in mean PaO$_2$/FiO$_2$ after the administration of rSPC. Treatment with this surfactant resulted in no significant change in PaO$_2$/FiO$_2$ in the control group compared with their respective pretreatment values. As observed in previous experiments, however, rSPC administration resulted in an increase in oxygenation in the lavaged group shortly after instillation, but this response deteriorated over the subsequent ventilatory period. In the NNMU-injured group, there was no change in oxygenation, whereas a deterioration in PaO$_2$/FiO$_2$ was observed in the HCl-injured group after surfactant administration. Figure 8 illustrates the surfactant pool sizes

![Fig. 4. Total and LA surfactant pool sizes in animals receiving bLES. Animals were mechanically ventilated for 1 h after intratracheal instillation of bLES and subsequently lavaged (n = 6/group). Total surfactant pool sizes did not differ among experimental groups. However, proportion of LA (%LA) in total pool was significantly lower in NNMU- and HCl-injured groups than in control group. Values are means ± SE. *Statistical significance (P < 0.05) vs. control.](image)

![Fig. 5. Western immunoblot identification of SP-A in LA fractions recovered from animals receiving bLES. SP-A was identified in samples of LA fraction (2.5 µg PL, n = 3/group) recovered from each experimental group by Western blot analysis and chemiluminescence technique. More SP-A was present in LA fraction recovered from control and NNMU-injured groups than from lavaged and HCl-injured groups.](image)

![Table 4. Total protein recovery and biophysical analysis of LA recovered from animals receiving bLES](table)

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Control</th>
<th>Lavaged</th>
<th>NNMU</th>
<th>HCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein recovery, mg/kg</td>
<td>6</td>
<td>81 ± 5</td>
<td>107 ± 7</td>
<td>349 ± 42*†</td>
<td>652 ± 26**†</td>
</tr>
<tr>
<td>Surface tension, mN/m</td>
<td>3</td>
<td>7.5 ± 2.8</td>
<td>19.6 ± 1.5</td>
<td>5.5 ± 1.4†</td>
<td>13.7 ± 3.2</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of animals. bLES, bovine lipid extract surfactant. *P < 0.05 vs. control; †P < 0.05 vs. lavaged; ‡P < 0.05 vs. NNMU.
recovered from these animals. Similar to the results obtained in the previous series of experiments, the total surfactant pool sizes did not differ significantly between the various groups of animals (n = 6/group); however, the percent LA was significantly lower in the HCl-injured group than in the other three groups (P < 0.01 vs. control, lavaged, and NNMU). Representative Western blots of LA and densitometric analyses of these LA revealed that samples isolated from the lavaged group had less SP-A (12% of control) than the other groups when identical quantities of PL were analyzed (for NNMU, 200% of control; for HCl, 58% of control).

Fig. 6. Surface area cycling of LA recovered from animals receiving bLES. LA samples (0.25 mg PL/ml) from each experimental group (n = 4/group) were cycled for 3 h at 37°C. Samples were analyzed as described in MATERIALS AND METHODS. LA fractions recovered from NNMU- and HCl-injured groups were converted to small aggregate (SA) fractions to a significantly lesser degree than LA samples recovered from control and lavaged groups. Values are means ± SE. Letter(s) represent statistical significance (P < 0.05) vs. control (a) and lavaged (b).

Fig. 7. Temporal changes in PaO2/FI02 after administration of recombinant surfactant-associated protein C drug product (rSPC). Arterial blood gas measurements were obtained at specified intervals during 60 min of ventilation after surfactant administration (n = 6/group). No changes in oxygenation were observed in control group over the ventilatory period. A rapid yet transient rise in oxygenation was observed in the lavaged group, but this deteriorated over the period of ventilation. No increase in PaO2/FI02 was observed in NNMU-injured group after surfactant administration. HCl-injured group demonstrated a rapid deterioration in oxygenation over course of ventilation. All animals were ventilated on an FI02 of 1.0. Values are means ± SE. *Statistical significance (P < 0.05) vs. control.

Fig. 8. Total and LA surfactant pool sizes in animals receiving rSPC. Animals were ventilated for 1 h after administration of rSPC and subsequently lavaged (n = 6/group). Total surfactant pool sizes did not differ between experimental groups. Proportion of LA in total pool (%LA) was significantly lower in HCl-injured group than in control group. Values are means ± SE. *Statistical (P < 0.05) significance vs. control.

Fig. 9. Table 5 shows the total protein recovery in the whole lung lavage obtained from these animals after death. Again, similar to animals given bLES, the greatest amount of protein was recovered from the HCl-injured animals (P < 0.001 vs. other groups), although protein recovery was increased in the lavaged and NNMU-injured animals compared with control animals (P < 0.05). The surface tension values of the LA recovered from these animals are also shown in Table 5. Surface tension values were not significantly different between the control, lavaged, and NNMU-injured groups; however, surface tension values were significantly lower in the lavaged group than in the HCl-injured group (P < 0.05). Figure 10 illustrates the in vitro conversion characteristics of the LA recovered from animals given rSPC. The conversion of native rSPC to SA was 18 ± 3% after 3 h. Interestingly, LA recovered from the lavaged group of animals converted to a greater extent than that recovered from the other groups (P < 0.02 vs. control, NNMU, and HCl). Less than 5% conversion to SA was observed when identical LA samples were incubated at 37°C but did not undergo surface area cycling (data not shown).

DISCUSSION

The efficacy of exogenous surfactant administered to patients with ARDS is variable. Although factors such as the timing of surfactant administration over the course of the injury as well as different surfactant delivery methods have been assessed in preclinical studies (17, 23), the significance of the specific type of lung injury in the efficacy of exogenous surfactant has not been formally addressed. Results of the present study showed that the characteristics of the underlying lung injury may influence the fate of an administered exogenous surfactant. However, in the clinical situa-
tion the nature of the endogenous environment cannot
be easily predicted or manipulated, so one may ulti-
mately need to tailor the choice of an exogenous surfac-
tant preparation to the type of lung injury present at
the time of treatment.

A variety of conditions may lead to ARDS (25), each
resulting in distinct changes in the endogenous surfac-
tant system at the time of treatment. The present study
was designed to characterize the fate of exogenous sur-
factant preparations when administered to four
different alveolar environments in vivo. Although the
term “environment” is relatively nonspecific, inasmuch
as a variety of components are present within the
alveolar space in an injured lung, we have specifically
focused on the endogenous surfactant system for this
study. The results of the first series of experiments
demonstrated different surfactant pool sizes, different
percentages of LA forms within the alveolar space, and
different levels of SP-A recovered in these four groups
of animals. There were also significant differences in the
total protein recovered in the lung lavage for the
various groups. Although the lavaged group was obvi-
ously a surfactant-depleted environment, the NNMU-
injured animals represented a more indirect pulmo-
nary insult initiated by the subcutaneous injection of
nitrosourea 48–72 h earlier. Clinically, this type of lung
injury was somewhat similar to that in patients with
sepsis-induced ARDS with respect to the relative time
course of the injury and the particular alterations in
endogenous surfactant observed. Analysis of the lung
lavage obtained from patients with sepsis-induced
ARDS included changes in PL and surfactant protein
composition, a decreased proportion of LA within the
air space, and abnormal surface activity of the isolated
surfactant (9, 32). These changes were very similar to
those observed in the NNMU-injured animals in the
present study. In addition to indirect lung injuries,
patients may also develop lung injury from a more
direct pulmonary insult, such as aspiration of gastric
contents or inhalation of toxic fumes. This condition
was mimicked by the HCl-induced injury utilized in
this study. Characterization of endogenous surfactant
in these types of patients has not been as extensive,
although our results showed that the surfactant alter-
ations in the HCl group were distinct from those in the
other groups. In general, therefore, the groups of
animals evaluated in this study represented four dis-
tinct endogenous surfactant environments. The physi-
ological parameters of these four groups of animals at
the time of surfactant administration were also differ-
ent (Table 5). Data from preliminary experiments indi-
cated that these particular physiological values were
necessary to ensure animal viability within each group
over the subsequent duration of the study. This factor,
as well as the relatively low doses of exogenous surfac-
tant utilized in this study, makes interpretation of the
physiological responses to these surfactants problem-
atic. As one would predict, oxygenation responses in the
lavaged group given exogenous surfactant were supe-

Table 5. Total protein recovery and biophysical
analysis of LA recovered from animals receiving rSPC

<table>
<thead>
<tr>
<th>n</th>
<th>Control</th>
<th>Lavaged</th>
<th>NNMU</th>
<th>HCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/kg</td>
<td>16 ± 2</td>
<td>101 ± 22</td>
<td>350 ± 29*</td>
<td>564 ± 27††</td>
</tr>
<tr>
<td>mN/m</td>
<td>15.1 ± 1.3</td>
<td>12.0 ± 0.6</td>
<td>18.7 ± 3.7†</td>
<td>21.8 ± 0.9</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of animals. rSPC, recombinant surfactant-associated protein C drug product. *P < 0.05 vs. control; †P < 0.05 vs. lavaged; ‡P < 0.05 vs. NNMU.
rior to those in the other lung injury groups. The more complex and potentially more severe lung injuries associated with the NNNU- and HCl-injured groups would no doubt require administration of higher doses of surfactant or alternative delivery strategies (2). A formal assessment of these factors was beyond the scope of the present study.

In addition to observing the obvious differences in the endogenous surfactant systems in these groups of animals, it is also important to consider the different mechanisms responsible for these alterations. These same mechanisms may influence the fate of an exogenously administered surfactant once it is deposited within the air space. For example, data obtained from the NNNU-injured rabbits in the present study were consistent with previous reports that showed that an increased conversion of LA to SA accounted for the decreased proportion of LA in these animals (33, 35). A relative decrease in superior-functioning LA surfactant pools has been shown to contribute to surfactant dysfunction and, consequently, lung dysfunction (20, 33). Inactivation of surfactant by serum proteins (13) has also been shown to cause surfactant dysfunction and no doubt also played a role in the pathophysiology of the lung dysfunction noted in these groups of animals.

When an exogenous surfactant was administered, further differences in surfactant aggregate forms and surfactant function were observed among groups. Although LA pool sizes and amounts of serum protein recovered from the air spaces 1 h after surfactant administration were similar in the NNNU- and HCl-injured groups receiving bLES, the in vitro surface activity of the LA fraction in the NNNU-injured group was superior to that of the LA fraction in the HCl-injured group. Furthermore, the biophysical function of the LA fraction in the NNNU-injured group was also superior to that in the lavaged group recovered after bLES administration. Again, this finding was observed, despite greater quantities of protein recovered in the former group than in the latter. These findings suggest that the functional differences in LA between these groups were related to the compositional differences of the recovered LA forms. Greater amounts of immunoreactive SP-A were associated with the LA isolated from the NNNU-injured animals than with the LA isolated from the lavaged and HCl-injured groups. SP-A may also play an important role in improving the function of these LA during our biophysical analysis.

A recent report by Ikegami et al. (15) characterized the endogenous surfactant isolated from SP-A-deficient mice. They showed a decreased proportion of LA in the SP-A-deficient mice compared with the wild-type mice, as well as an increased sensitivity of these LA to plasma protein inhibition. These as well as other reports suggest that SP-A is an important factor in maintaining LA integrity and enhancing the biophysical activity of surfactant, particularly in the setting of lung injury. Interestingly, the in vivo function of surfactant isolated from the SP-A-deficient and wild-type mice was similar, although these studies were conducted in a surfactant-deficient rabbit model, and the function was assessed over a relatively short period of time (15 min after administration). The relevance of changes in alveolar SP-A levels in different types of lung injury is unknown, although previous studies as well as our results suggest that the association of endogenous SP-A with exogenously administered surfactant may influence the metabolism and efficacy of these preparations.

The interaction, or at least association, of SP-A with an exogenous surfactant has previously been suggested in preterm lambs and NNNU-injured adult rabbits (16, 29). In these two studies the in vitro and in vivo function of alveolar surfactant isolated from the animals after administration of exogenous surfactant was superior to that after the actual surfactant preparation was delivered. The authors of these studies postulated that it was the incorporation of endogenous SP-A into the exogenous preparations that was responsible for their findings. In our study, since bLES contained no SP-A, this protein in the LA forms at death was from endogenous sources. Moreover, similar to previous reports (16), the greater amounts of SP-A detected in the LA samples isolated from the NNNU-injured animals after bLES administration were associated with superior in vitro function of these samples. The mechanisms responsible for the functional superiority of SP-A-containing LA may be due to an increased resistance of these aggregate forms to protein inactivation (4). In general, therefore, our results suggest that, in patients with ARDS, it is important not only to consider the changes in total surfactant pool sizes of the various aggregate forms but also the functional activity of these aggregates. Both factors may contribute to the lung dysfunction associated with ARDS.

In the present study, several metabolic processes may have contributed to the different amounts of LA recovered from the groups of animals receiving bLES. These processes include altered tissue uptake of surfactant, changes in the secretion or recycling of surfactant components, or, as noted previously, differences in the conversion of LA to SA (21). Because we observed no significant differences in total surfactant pool sizes between these groups, it is unlikely that different secretion and/or recycling kinetics occurred over this short period of time. The observed differences in LA pool sizes were likely due to altered aggregate conversion. LA conversion has been shown to be influenced by many factors, including the amount of SP-A present and the inherent stability of the LA themselves (12, 34). To provide further insight into the metabolic stability of the LA forms isolated from these various groups, in vitro surface area cycling experiments were performed (11, 12) (Figs. 4 and 8). The conversion properties of LA forms obtained from bLES-treated animals differed among groups and were significantly lower than the conversion characteristics of bLES. This finding was consistent with the hypothesis that some component(s) of the alveolar environment, potentially SP-A, influenced LA conversion, which accounted for the differences in surfactant aggregate forms observed in the four groups of animals. However, these in vitro conver-
sion properties may not entirely reflect the conversion characteristics of LA forms in vivo. Additional in vivo studies are required to further assess the specific factors within the alveolar environment that may have contributed to the differences in aggregate pool sizes noted between these groups of animals.

Results of the final series of experiments involving rSPC revealed that the interaction of endogenous surfactant components with exogenous surfactant preparations is also dependent on the characteristics of the exogenous surfactant preparation administered. The main difference between bLES and rSPC surfactants was the absence of SP-B in the latter preparation. Previous studies have shown that SP-A may associate and form tubular myelin preferentially with lipid mixtures containing SP-B rather than SP-C (36). In the present study the in vitro surface tension values of the LA recovered from the bLES-treated groups were lower than those of the LA recovered from the rSPC-treated group, particularly in animals with alveolar environments containing SP-A. For example, LA isolated from the NNMU-injured animals given rSPC had inferior surface activity compared with the LA isolated from NNMU-injured animals given bLES (P < 0.05). On the other hand, the lavaged animals had very little SP-A available for association with either exogenous surfactant preparation; thus the biophysical activities of the LA obtained from these two groups of animals were similar to the biophysical activities of the respective exogenous surfactant preparations when tested in vitro (6). On the basis of this information, we speculate that the use of a surfactant preparation containing SP-B may be preferred in an alveolar environment containing greater quantities of SP-A because of the potential for this protein to associate and interact with the exogenous surfactant preparation.

In summary, we have shown that the nature of the endogenous surfactant system influenced the metabolic and biophysical properties of an exogenous surfactant once it was deposited within the air space. We speculate that this factor may be important to consider when exogenous surfactant treatment strategies are designed for patients with ARDS. The various components of the alveolar environment responsible for the effects on exogenous surfactant require further characterization, although it appears that SP-A is at least one important factor to consider. In clinical terms, this may require sampling the alveolar space before surfactant treatment and choosing a specific exogenous surfactant on the basis of these results.

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