Characterizing alterations in the pulmonary surfactant system in a rat model of *Pseudomonas aeruginosa* pneumonia


**ABSTRACT:** Bacterial pneumonia remains a significant cause of patient morbidity and mortality worldwide. Pulmonary surfactant serves to maintain homeostasis in the lung through the maintenance of alveolar stability and the regulation of the alveolar immune response. The purpose of this study was to characterize the lung injury and associated surfactant alterations in a rat model of acute *Pseudomonas aeruginosa* pneumonia.

Pneumonia was induced in male Sprague–Dawley rats via intratracheal injection of 0.2 mL phosphate-buffered saline (PBS) containing *P. aeruginosa* (6×10⁸ colony-forming units mL⁻¹). Control animals received 0.2 mL sterile PBS.

Twenty-four hours after inoculation, the pneumonia group (PN) exhibited clinical signs of pneumonia including deficits in gas exchange, leukopenia and elevated arterial lactate levels. Morphological assessment confirmed the presence of pneumonia with airspace filled with polymorphonuclear cells. Lung homogenate analysis demonstrated evidence of bacterial colonization of pulmonary lung tissue. Lung compliance was also significantly lower in the PN group. Lung lavage analysis of PN rats revealed the pooled surfactant levels to be lower and the surfactant function reduced compared to control rats. Surfactant composition was also found to be altered in PN rats.

These results demonstrate that in *Pseudomonas aeruginosa* pneumonia, the pulmonary surfactant system is both poorly functioning and reduced in quantity. These alterations may contribute to the lung dysfunction characteristic of this disorder.
this study, therefore, was to establish a clinically relevant animal model of acute P. aeruginosa pneumonia and characterize the alterations in the pulmonary surfactant system in this model. This information will provide further insight into the role of the surfactant system in bacterial pneumonia and may ultimately lead to treatment strategies involving exogenous surfactant.

Materials and methods

Animals

Adult male Sprague–Dawley rats (Charles River, Constant, Quebec, Canada), weighing approximately 350–450 g, were used for these experiments. Experimental procedures complied with standards outlined by the Canadian Council on Animal Care and the Animals for Research Act of Ontario.

Study design

Rats were intratracheally instilled with a bacterial suspension of P. aeruginosa. Control rats were instilled with a similar volume of sterile saline. Twenty-four hours after inoculation, arterial blood gases and blood cell counts were measured and rats were sacrificed for subsequent measures of lung compliance and the analysis of the pulmonary surfactant system. Surfactant quantity, composition and function were characterized. Separate animals were studied for morphological assessment and bacterial colonisation of lung tissue.

Preparation of bacterial inoculum

Each volume of inoculum of P. aeruginosa (ATCC strain 27853) was prepared fresh daily from an overnight culture plate and resuspended in sterile phosphate-buffered saline (PBS), pH 7.2. The optical density from the culture plate and resuspended in sterile phosphate-buffered saline (PBS), pH 7.2. The optical density from the culture plate and resuspended in sterile phos-

Animal preparation

Rats were acclimatized to the laboratory environment for 1 week before experimentation. During this acclimati-
rats were killed by transection of the descending aorta. Rats then underwent either lung compliance measurements with a subsequent whole-lung lavage for surfactant analysis or a whole-lung homogenization for bacterial counts.

**Morphological assessment**

Three animals within each group were studied for morphological assessment, with the pathologist blinded to the rat’s experimental condition. The heart and lungs were removed en bloc from the animals and immediately placed in 10% neutral-buffered formalin fixative. Inflation of the lungs was achieved by tracheobronchial perfusion using a 22-gauge butterfly needle connected to a syringe filled with 5 mL formalin. Following overnight fixation, the lobes of the lungs were separated and grossly examined and representative sections were taken from each lobe for histological evaluation. The sections were embedded in paraffin and 5 µm sections were stained with haematoxylin and eosin.

**Lung compliance measures**

In separate groups of rats, immediately after the animals were killed, the chest wall was removed in order to visualize both lungs and a tracheostomy was performed for endotracheal tube insertion. Rats were then placed in a vacuum chamber for approximately 1 min to evacuate residual air within the lungs. Once removed from the chamber, the endotracheal tube was attached to a U-shaped manometer and the lungs were inflated in 2 cmH₂O pressure increments using a glass syringe. A 20 s pause was performed at each pressure interval to ensure adequate filling. The corresponding volumes administered were recorded from the syringe at each pressure, up to a maximum pressure of 26 cmH₂O. Lungs were then deflated in the same pressure increments to a minimum pressure of 0 cmH₂O.

**Lung lavage analysis**

Ten millilitres of 0.9% NaCl was infused into the lungs via the endotracheal tube, withdrawn and reinfused twice. This procedure was repeated a total of four times with the final total volume of crude alveolar wash (CAW) recorded [20]. Ten millilitres of the CAW was stored for total surfactant pool size analysis, surfactant-associated protein measurements and total protein measurements. The remainder of the CAW was centrifuged in order to separate the alveolar surfactant into different aggregate subfractions as described previously [21, 22]. In brief, the CAW sample was centrifuged for 10 min at 150,000×g yielding a pellet (P1) containing primarily cellular debris. The resulting supernatant was then centrifuged for 15 min at 40,000×g yielding a pellet containing the large aggregate (LA) surfactant subfraction and a supernatant containing the small aggregate (SA) surfactant subfraction. P1 and LA pellets were each resuspended in 2.2 mL 0.9% NaCl for further analysis.

Aliquots of CAW, P1 pellet, and LA and SA fractions were extracted using the chloroform–methanol procedure described by Buck-Chong [23]. This procedure was performed in order to isolate lipids and remove any proteins, phospholipases or proteases from the lavage which may have interfered with our subsequent surfactant quantification procedures. Following this extraction step, samples were then dried under nitrogen and disaturated phospholipids (DSPL), the main functional lipid component of surfactant, were isolated and measured as described previously [24]. In brief, 0.5 mL 0.5% (w/v) osmium tetroxide in CCl₄ was added to the dried sample tube, followed by an additional period of drying under nitrogen. A chloroform–methanol (20:1) solution was used to wash an alumina column (Sep-Pak Alumina N Cartridges; Waters, Millford, MA, USA). This solution was then added to the samples and passed through the washed column. The DSPL component was eluted from the column using a chloroform–methanol–ammonium hydroxide solution (35:15:1, v/v/v).

The total DSPL phosphorus content of each eluent was then quantified using a modification of the method described previously by Duck-Chong [25]. The total phosphorus contents of each of the CAW, P1, as well as the LA and SA-containing subfractions were also measured before the DSPL isolation procedure using a similar method. In brief, 100 µL 10% magnesium nitrate in methanol was added to the samples. Samples were then dried under air and subsequently ashed on an electric rack for approximately 1 min. Ashing was followed by the addition of 1 mL 1 M HCl and subsequent heating for 15 min at 95°C. After cooling, a 66-µL aliquot of each sample was added to the well of a 96-well plate and 134 µL of a dye reagent consisting of 4.2% ammonium molybdate in 4.5 M HCl with 0.3% malachite green was added. Sample absorbance at 650 nm was then measured using a MKII Titretek Multiskan enzyme-linked immunosorbent assay (ELISA) plate reader and compared with reference standards. The total CAW protein content was quantified using the method of Lowry et al. [26] using bovine serum albumin (BSA) as a standard.

**Assessment of surface activity**

Aliquots of the isolated LA surfactant fraction were resuspended in 1.5 mM CaCl₂ in 0.9% saline to a concentration of 1 mg phospholipid·mL⁻¹. Surface activity was assessed using a pulsating bubble surfactometer (Elecnotics Corporation, Buffalo, NY, USA) as described previously [27]. In brief, a bubble was created in the suspension containing the LA surfactant fraction. After 10 s of adsorption, the bubble was pulsed for a period of 5 min between a maximum radius of 0.55 mm and a minimum radius of 0.44 mm at a rate of 20 pulsations-min⁻¹ and a temperature of 37°C. Pressure was monitored across the air–liquid interface using a pressure transducer and surface tensions at the minimum bubble radius (Rmin) were calculated.

**Surfactant protein measures**

A previously established ELISA [20, 22, 28, 29] was used to measure rat surfactant-associated protein (SP)-A
and rat SP-B in the CAW of both control and PN animals. For SP-A, wells were coated with a 1/100 dilution of concentrated mouse lavage in 0.1 NaHCO₃. Wells were blocked with 5% human serum in 82b (0.15 M NaCl, 0.01 tris-hydroxymethyl-amino methane (Tris), pH 7.4, and 5 µg-mL⁻¹ of BSA). Samples and standards were preincubated overnight at 37°C in a 1/8,000 dilution of rabbit antiserum to SP-A in 82b with 5% human serum and 0.5% Nonidet P-40. The samples and standards were then incubated in the coated wells for 1–2 h, and the wells were washed and incubated with goat antirabbit immunoglobulin (IgG) conjugated to peroxidase (Calbiochem, La Jolla, California, USA). After washing, colour was developed using 100 µL 0.065 M NaKPO₄, pH 6.3, containing 0.017 M citric acid, 0.05% H₂O₂, and 5 mg-mL⁻¹ o-phenylenediamine. The colour reaction was stopped using 50% sulphuric acid and the absorbance at 492 nm was determined.

For SP-B determination, wells were incubated with standard purified bovine SP-B (0.5–10 ng) or sample, a polyclonal antirabbit SP-B antibody and a secondary antirabbit IgG antibody conjugated to peroxidase. All samples were analysed simultaneously in a single assay. Colour was developed and measured as described above for SP-A.

**Phospholipid composition**

Using a separate group of animals, total CAW samples were centrifuged for 10 min at 150g and cellular debris was removed. The resulting supernatant was extracted using the method described by BLIGH and Dyer [23] and dried slowly under nitrogen. The remaining pellet was dissolved in 2 mL chloroform. Phosphorus measurements were made on aliquots of the chloroform solution using a modification of the method described by Dvuck-Chong [25]. Subsequently, samples were dried under nitrogen and the pellet was dissolved in chloroform to a total phospholipid concentration of 0.2 mg·mL⁻¹. Phospholipid compositions were performed using thin-layer chromatography (TLC) as described previously [22]. In brief, 50 µL aliquots of the sample and 5 µL aliquots of reference standards were spotted on linear-K silica gel TLC (Whatman, Maidstone, UK) plates and developed using an ethanol–triethylamine–chloroform–water developing solvent (35:34:30:8, v/v/v/v). After reference standards were visualized with Dittmer–Lester phosphorus spray [30], the corresponding phospholipids were scraped from sample lanes and phosphorus was measured using the Dvuck-Chong assay [25].

**Bacterial counts**

The whole lungs of animals used for bacterial analysis were aseptically removed en bloc from the chest cavity and the trachea and large airways were dissected. The remaining lung tissue was then weighed and suspended in 3 mL sterile 10 mM HEPES buffer containing 0.32 M sucrose. Using a T25 rotor-stator tissue homogenizer (Janke and Kunkel, Staufen, Germany), the lungs from each animal were homogenized and the total volume of lung homogenate was recorded. Serial 10-fold dilutions using 0.9% NaCl were prepared in duplicate from the original lung homogenate fraction, up to a dilution factor of 1:10,000. Then, 50-µL aliquots of each dilution were then plated in duplicate on 7% sheep blood agar and incubated at 35°C for 24 h. Following incubation, the culture plates were examined for bacterial growth and pseudomonas colonies were visually identified and counted. An average from the two plates was determined and, using the dilution factor, the total bacterial load in the lungs was calculated and expressed as percentage recovery from the initial input. All materials used in the above procedure were sterile.

**Data analysis**

The alveolar–arterial oxygen tension gradient (Pₐ-aO₂) was found using the alveolar gas equation to determine alveolar oxygen tension (PₐO₂): PₐO₂ = (Pb - PₐH₂O) × FLo₂ - (PₐCO₂/R), where Pb is barometric pressure, PₐH₂O is the partial pressure of water vapour, FLo₂ is the inspired O₂ fraction, PₐCO₂ is the mean alveolar carbon dioxide tension (assumed to be equal to arterial CO₂ tension), and R is the respiratory quotient (assumed to be equal to 0.8).

All data are expressed as means±SEM. Values obtained from control and PN groups were compared using an unpaired Student’s t-test (two-tailed) and a probability level of p<0.05 was considered to be statistically significant.

**Results**

**Physiological parameters**

Table 1 shows mean values for body weight, Pₐ-aO₂, fC, fR, MAP, arterial lactate and white blood cell counts (WBC) for both the control and PN groups 24 h after tracheal instillation. There was significantly altered gas exchange in the pneumonic animals compared with the control animals. (1 mmHg=0.133 kPa.)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>PN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight</td>
<td>388±8</td>
<td>395±7</td>
</tr>
<tr>
<td>PaO₂ mmHg</td>
<td>83.6±2.5</td>
<td>69.8±1.6*</td>
</tr>
<tr>
<td>Pa-aO₂</td>
<td>16.6±1.7</td>
<td>29±1.7*</td>
</tr>
<tr>
<td>fR breaths·min⁻¹</td>
<td>97±3</td>
<td>120±5*</td>
</tr>
<tr>
<td>fc beats·min⁻¹</td>
<td>333±8</td>
<td>323±7</td>
</tr>
<tr>
<td>MAP mmHg</td>
<td>118±3</td>
<td>106±2*</td>
</tr>
<tr>
<td>Lactate mmol·L⁻¹</td>
<td>0.50±0.027</td>
<td>0.67±0.034*</td>
</tr>
<tr>
<td>WBC ×10⁴ cells·mL⁻¹</td>
<td>11.5±1.2</td>
<td>6.6±0.8*</td>
</tr>
</tbody>
</table>

**Table 1.** — Physiological parameters of sham-treated control and pneumonic (PN) rats 24 h after intratracheal instillation of sterile phosphate-buffered saline and *Pseudomonas aeruginosa*, respectively.

Values are means±SEM. Numbers in parentheses are numbers of rats. PaO₂: arterial oxygen tension; Pa-aO₂: alveolar–arterial oxygen tension gradient; fR: respiratory frequency; fc: cardiac frequency; MAP: mean arterial pressure; WBC: white blood cell counts*: p<0.05 versus control animals. (1 mmHg=0.133 kPa.)
control rats. This was characterized by lower arterial oxygen tension ($P_{a,O_2}$) values, elevated $P_{A-a,O_2}$ gradients and higher $f_R$ in the PN group ($p<0.05$). There were no significant differences in $P_{a,CO_2}$ values between the two groups (data not shown).

Modest haemodynamic changes were also evident in the PN group compared with the control group just before killing. Although $f_C$ was comparable between groups, the MAP was slightly, albeit significantly, lower in the pneumonia group ($p<0.05$). PN rats also had significantly higher lactate levels than control rats and significantly lower WBC counts ($p<0.05$).

**Morphological assessment**

The morphological appearance of lungs from animals in both the control and PN groups are shown in figure 1. The control animals showed normal lungs with no evidence of injury. In PN animals, there was evidence of acute bronchopneumonia with the airspaces filled with abundant polymorphonuclear cells, some red cells, and fibrin.

**Pressure–volume curves**

Static lung compliance was determined via measurements of pressure–volume curves after killing (fig. 2). This graph shows that the lung compliance of PN rats was significantly different to the compliance measured in the control rats. The maximum recruitable volume ($V_{\text{max}}$) at the highest inflation pressure (26 cmH$_2$O) was significantly lower in the PN rats ($p<0.05$). On the deflation limb of the curve, the volume remaining in the lungs at a pressure of 6 cmH$_2$O ($V_6$) was also significantly lower in the PN group than in control animals ($p<0.05$), as were values at each transpulmonary pressure on the deflation limb of the curve. In addition, a decreased hysteresis was observed in rats instilled with pseudomonas compared with the control animals.

**Surfactant analysis**

The total phospholipid pool sizes of CAW, cellular pellet (P1), and both LA and SA surfactant fractions were determined.
intratracheal instillation of sterile phosphate-buffered saline or Pseudomonas aeruginosa, respectively. P1 is the subfraction containing primarily cellular debris. The total DSPL content of CAW, as well as the DSPL content of the large aggregate (LA) subfraction, was significantly lower in PN rats than in control rats (*: p<0.05).

**Table 2.** Surfactant-associated protein levels and phospholipid composition of surfactant obtained from sham-treated control and pneumonic (PN) rats 24 h after intratracheal instillation of sterile phosphate-buffered saline and Pseudomonas aeruginosa, respectively.

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>%PC</th>
<th>%PG</th>
<th>%SM</th>
<th>%PE</th>
<th>%LPC</th>
<th>%PI+PS</th>
<th>PC/PI+PS</th>
<th>PG/PI+PS</th>
<th>SP-A (µg·kg⁻¹)</th>
<th>SP-B (µg·kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham (6)</td>
<td>75.2±3.0</td>
<td>3.5±0.5</td>
<td>14.3±3.2</td>
<td>1.5±0.3</td>
<td>4.2±0.3</td>
<td>4.0±0.1</td>
<td>18.1±0.7</td>
<td>0.9±0.1</td>
<td>75.8±16.3</td>
<td>4.1±0.5</td>
</tr>
<tr>
<td>PN (6)</td>
<td>73.6±2.2</td>
<td>2.9±0.6</td>
<td>12.1±1.3</td>
<td>1.0±0.2</td>
<td>3.9±0.7</td>
<td>6.5±0.9</td>
<td>12.5±1.7*</td>
<td>0.5±0.1*</td>
<td>59.7±10.8</td>
<td>4.4±0.4</td>
</tr>
</tbody>
</table>

Values are means±SEM. %: percentage of total phospholipid pool. PC: phosphatidylcholine; PG: phosphatidylglycerol; SM: sphingomyelin; PE: phosphatidylethanolamine; LPC: lysophosphatidylcholine; PI+PS: phosphatidylinositol+phosphatidylserine; SP-A: surfactant-associated protein A; SP-B: surfactant-associated protein B. *: p<0.05 versus control.
of lung tissue was evident by gross inspection, only a small percentage (0.07±0.02%) of the initial bacterial input (1.0±1.3×10^7 cfu·mL⁻¹) was recovered in the lung homogenate at 24 h.

Control experiments were conducted in which lungs were homogenized immediately after inoculation with pseudomonas. Similarly, the same pseudomonas inoculum was added directly to the homogenate of noninfected lungs. In both cases, approximately 50% of the initial bacterial input was recovered, indicating that the low recovery at 24 h in the PN group was primarily due to host clearance.

Discussion

_P. aeruginosa_ is the infectious organism responsible for most cases of nosocomial pneumonia [1]. Presently, the treatment for this disorder consists primarily of antibiotic administration, although typical antibiotic combinations have had little effect on patient morbidity and mortality [1, 2].

In this study, a clinically relevant rat model of acute pseudomonas pneumonia was established, characterized by physiological alterations similar to those observed in patients with this illness. These impairments included disturbances in gas exchange reflected by arterial hypoxaemia and a significant elevation in PaO₂, elevated respiratory rates and a decrease in lung compliance, reflected by changes in pressure–volume curves. MAP was also slightly reduced in the infected animals, and although this reduction was statistically significant and has been shown in other animal models of pneumonia [31, 32], the actual pressures measured in these animals remained essentially normotensive and did not result in a hypoperfused state. Other changes characteristic of the bacterial infection induced in these animals included elevated arterial lactate levels, leukopenia and morphological evidence of bronchopneumonia with airspaces filled with abundant polymorphonuclear cells. These latter morphological changes, together with the recovery of _P. aeruginosa_ bacteria from lung tissue at the time of killing, confirm the presence of pneumonia in these animals and are very similar to the changes seen in patients with bronchopneumonia.

Associated with the observed physiological changes, and no doubt contributing to these changes, were the observed alterations in the endogenous surfactant system. Previous studies analysing BAL samples obtained from patients with pneumonia [16, 33] noted a decrease in phosphatidylcholine concentrations. Similar observations were noted in the present study, which saw a decrease in the lavage-obtained DSPL pool of pneumonia animals, of which dipalmitylphosphatidylcholine is the major component. King et al. [34] reported a 33% decrease in total surfactant phospholipid concentrations in adult baboons following hyperoxia and _P. aeruginosa_-induced lung injury. Similarly, Tavolga and Hallock [35] observed a 52–67% decrease in total surfactant phospholipids following endotracheal injection of endotoxin in guinea-pigs. One limitation of these previous studies, however, was the technique used to sample the alveolar space. For example, BAL samples only reflect changes occurring in the area from which material was obtained and are only capable of measuring surfactant phospholipid concentrations rather than total pool sizes. The whole lung lavage method used in the present study permitted a very accurate measurement of total surfactant pool sizes. The results confirmed that, by the observed decrease in DSPL levels (the major components of surfactant), the total quantity of functional alveolar surfactant was decreased in PN animals compared with control animals.

An additional novel finding in the present study was that there was a decrease in the LA surfactant fraction in the PN animals, which was probably responsible for the observed changes in the total surfactant pool size. This was an important finding as LA forms of surfactant have been shown to be primarily responsible for surface tension reduction at the air–liquid interface [21]. Indeed, reports from experimental animal models of acute lung injury reflecting the clinical disease of ARDS have also reported a relative decrease in the surfactant LA fraction, as well as an increase in the SA fraction [21, 22, 36]. In these studies, the aggregate changes were shown to contribute directly to the decreased lung compliance and decreased PaO₂ values observed in the injured animals [17, 22]. Based on these observations, it was speculated that the decrease in the proportion of LA observed in the PN animals contributed to the abnormal physiology demonstrated in these animals.

In addition to the decreased quantity of the LA fraction recovered in the PN animals, there was a significant impairment in the function of the LA fractions. LA isolated from the PN rats did not reduce surface tension to the levels observed for LA isolated from control rats. This finding was consistent with functional assessments of LA isolated from animals with acute lung injury [17, 36]. Although previous functional impairments of BAL samples obtained from patients with pneumonia have been demonstrated [16, 33], this study has shown that it is the LA fraction in particular that is responsible for the surfactant dysfunction in pneumonia.

The potential mechanisms responsible for this loss of functional activity of the endogenous surfactant system may be several-fold. Firstly, the inhibitory effects of bacterial endotoxin and its lipopolysaccharide constituents on pulmonary surfactant have previously been shown to be important in in vitro studies [33]. Secondly, it is likely that the leakage of plasma proteins into the alveolar space [37] contributed to surfactant inhibition in PN rats, as this phenomenon has previously been shown to contribute to surfactant dysfunction [17] in animal models of acute lung injury. Moreover, plasma protein leakage into the alveolar space of PN animals was confirmed by an observed increase in the total protein content of CAW obtained from these rats. This also probably reflects the protein content of surfactant LA fractions in these two groups. These LA preparations were tested for surface activity at a total phospholipid concentration of 1 mg·mL⁻¹, but measurements of DSPL revealed that the LA fraction of PN animals contained a smaller percentage of phospholipids in the disaturated form than did the LA fractions of the control animals. Although this difference did not reach statistical significance, the lower quantity of functionally active DSPL in the PN LA samples may have contributed to the surfactant dysfunction observed in this group. A combination of a higher quantity of protein and a lower quantity of DSPL in the PN LA fractions could explain the abnormal
functional activity of the isolated LA forms obtained from PN rats.

Other mechanisms may also have resulted in the decreased quantity of surfactant LA forms in the PN rats. While LA have been shown to be the metabolic precursors of SA and are continuously being converted into SA in the normal alveolar environment, under certain conditions (i.e. lung injury) this conversion process has been shown to be increased [17, 21]. For example, it has been suggested that in acute lung injury, an increase in protease activity results in an increased conversion of LA into SA and, therefore, a decrease in the size of the LA pool [17, 22, 36]. In this study, a similar process was probably occurring, although no increase in the SA pool size was observed. This may well have been due to an increase in SA uptake by alveolar type II cells, or an increase in clearance of SA forms by alveolar macrophages, a route of clearance of endogenous SA forms demonstrated in normal lungs [17]. Further studies are required to elucidate fully the mechanisms responsible for the changes observed in the aggregate pool sizes in these animals.

In contrast to previous observations in patients with pneumonia, no significant changes in the proportion of individual surfactant phospholipids were observed. Previous human studies reported decreased levels of PC and PG and increased levels of sphingomyelin and PI [16]. A potential reason for this discrepancy may be that these alterations are either species-specific or pathogen-specific, as the human studies involved a variety of different infectious agents [16]. Despite these discrepant results, changes were observed in phospholipid ratios. While this finding was consistent with previous reports of pneumonia [34, 38], it is of questionable functional importance as previous studies of acute lung injury have provided no evidence indicating that changes in phospholipid composition changes contribute significantly to lung dysfunction [17, 36].

Although phospholipid composition was altered in PN animals, there were no significant differences noted in surfactant-associated protein levels, again a finding in contrast to human studies. This discrepancy could be due to the specific pathogen being studied. While one study demonstrated increased SP-A levels in BAL samples from patients with Pneumocystis carinii pneumonia [39], other studies have shown decreased SP-A levels in patients with bacterial pneumonia [15, 16]. It should be noted that while there were no differences observed in the total quantity of recovered SP-A and SP-B between the PN and control animals in the present study, the function of these proteins was not assessed. Further studies addressing this issue may provide additional information that will aid our un-derstanding of the role played by these proteins in the course of bacterial infection.

The similarities of the surfactant alterations in both pneumonia and acute lung injury have been discussed. These observations may reflect the general inflammatory response associated with both conditions. It is also interesting to note that 70% of patients dying from ARDS were noted to have pneumonia upon post mortem examination [40]. These observations, and indeed the overall relevance of the present study, reflect the role of the pulmonary surfactant system in host defence. Traditionally, the major function of pulmonary surfactant has been assumed to be biophysical, namely lowering surface tension to maintain alveolar stability and prevent alveolar collapse. If the surfactant system is altered, the consequences are decreased lung compliance, alveolar oedema and arterial hypoxaemia. More recently, however, several in vitro studies have implicated surfactant as an important component of the inflammatory and infectious response, regulating the release of inflammatory mediators in the airspace, as well as enhancing the antibacterial activities of alveolar macrophages. In vivo, however, the role of surfactant in this ca-pacity is unknown, although the current model may prove valuable in assessing the role of surfactant in maintaining the integrity of host defence over the course of pneumonia.

In summary, a rat model of an acute pulmonary infection with Pseudomonas aeruginosa was developed, with the resulting pathophysiology typical of that observed in patients with bacterial pneumonia. Alterations to the endogenous surfactant system were also observed and these changes have been shown to contribute to the lung dysfunction associated with this condition. As antibiotic therapy is not always effective in the treatment of pneumonia and since the surfactant system has a role in the pathophysiology of this disorder, exogenous surfactant administration represents a potential therapeutic strategy, both from a physiological standpoint and in host defence. This model will ultimately serve as a valuable tool in assessing the efficacy of this potential treatment strategy.

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References
12. Van Iwaarden JF, Shimizu H, Van Golde PH, Voelker DR,
11. Weissbach S, Neuendank A, Pettersson M, Schaberg T,
10. Wright JR, Youmans DC. Pulmonary surfactant protein A


