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Exogenous pulmonary surfactant as a drug delivering agent: influence of antibiotics on surfactant activity

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1 It has been proposed to use exogenous pulmonary surfactant as a drug delivery system for antibiotics to the alveolar compartment of the lung. Little, however, is known about interactions between pulmonary surfactant and antimicrobial agents. This study investigated the activity of a bovine pulmonary surfactant after mixture with amphotericin B, amoxicillin, ceftazidime, pentamidine or tobramycin.

2 Surfactant (1 mg ml^{-1} *in vitro* and 40 mg ml^{-1} *in vivo*) was mixed with 0.375 mg ml^{-1} amphotericin B, 50 mg ml^{-1} amoxicillin, 37.5 mg ml^{-1} ceftazidime, 1 mg ml^{-1} pentamidine and 2.5 mg ml^{-1} tobramycin. Minimal surface tension of 50 μl of the mixtures was measured *in vitro* by use of the Wilhelmy balance. *In vivo* surfactant activity was evaluated by its capacity to restore gas exchange in an established rat model for surfactant deficiency.

3 Surfactant deficiency was induced in ventilated rats by repeated lavage of the lung with warm saline until PaO_2 dropped below 80 cmH_2O with 100% inspired oxygen at standard ventilation settings. Subsequently an antibiotic-surfactant mixture, saline, air, or surfactant alone was instilled intratracheally (4 ml kg^{-1} volume, $n=6$ per treatment) and blood gas values were measured 5, 30, 60, 90 and 120 min after instillation.

4 The results showed that minimal surface tensions of the mixtures were comparable to that of surfactant alone. *In vivo* PaO_2 levels in the animals receiving ceftazidime-surfactant or pentamidine-surfactant were unchanged when compared to the surfactant group. PaO_2 levels in animals receiving amphotericin B-surfactant, amoxicillin-surfactant or tobramycin-surfactant were significantly decreased compared to the surfactant group. For tobramycin it was further found that PaO_2 levels were not affected when 0.2 M $NaHCO_3$ (pH = 8.3) buffer was used for suspending surfactant instead of saline.

5 It is concluded that some antibiotics affect the *in vivo* activity of a bovine pulmonary surfactant. Therefore, before using surfactant-antibiotic mixtures in clinical trials, interactions between the two agents should be carefully evaluated.

Keywords: Pulmonary surfactant, antimicrobial agents, antibiotics, pneumonia, surfactant function, drug delivery, lung lavage model

Introduction

Efficient antimicrobial therapy is considered to be dependent on appropriate antibiotic concentrations at the site of infection (Baldwin *et al.*, 1992). For pneumonia this is within the alveolar space together with the epithelial lining fluid and the lung interstitium (Spencer, 1985). When administered systemically, it is difficult to ensure efficient concentrations of some antibiotics at the infection site without inducing severe adverse reactions, e.g. oto- and nephrotoxicity by aminoglycosides (McCormack & Jewesson, 1992). Methods for more selective delivery of antimicrobial agents to the lung and infected lung areas in particular seem, therefore, a potential way to increase therapeutic efficacy.

Application of antibiotics to the airways, either inhaled as an aerosol or injected directly into the trachea, has been studied almost since their discovery. Aerosols are, however, not deposited in non-ventilated lung areas (Laube *et al.*, 1989). Moreover, in patients with decreased pulmonary function, pulmonary deposition is particularly high in the central airways and decreases towards the periphery (Ilowite *et al.*, 1987; Laube *et al.*, 1989). With direct endotracheal instillation distribution is largely limited to the central airways (Brain *et al.*, 1976). Thus, the therapeutic efficacy of these administration modes seems limited, especially since the location of infection is more peripheral.

Tracheal instillation of exogenous pulmonary surfactant, a mixture of phospholipids and specific surfactant proteins, is an established therapy in neonates suffering from respiratory distress syndrome (Jobe, 1993). The excellent spreading properties of pulmonary surfactant within the lung suggest that exogenous surfactant could be exploited as a carrier for drug delivery to the alveolar compartment of the lung (Kharasch *et al.*, 1991; Schäfer, 1992; Lachmann & Gommers, 1993). It is shown by Kharasch *et al.* (1991) that tracheal instillation of a pentamidine-surfactant mixture marked with a radioactive colloid has a more uniform and wider distribution pattern in the lung than instillation of a pentamidine-saline solution.

Furthermore, it has been shown that instillation of pulmonary surfactant in infected lungs can improve gas exchange, restore lung function and re-expand atelectatic areas (van Daal *et al.*, 1991; Eijking *et al.*, 1991; van Daal *et al.*, 1992). It is expected that, mixed with the surfactant, efficient antibiotic dosages can be delivered even to the non-ventilated areas.

Little is known, however, about possible interactions between pulmonary surfactant and antimicrobial agents when mixed. A previous study (van 't Veen *et al.*, 1995) showed that the *in vitro* bactericidal activity of amoxicillin and ceftazidime was unaffected in the presence of pulmonary surfactant. However, activity of tobramycin was significantly reduced in the presence of pulmonary surfactant. These results demonstrated the relevance of studying antibiotic activity and surfactant activity when they are mixed.

The present study investigated surfactant activity after mixture with amphotericin B, amoxicillin, ceftazidime, pentamidine or tobramycin. These antibiotics, from different classes,

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were chosen on the basis of their clinical relevance in the treatment of lower respiratory tract infections in the intensive care unit (ICU). Minimal surface tension of antibiotic-surfactant mixtures was measured *in vitro* by use of a Wilhelmy balance and compared to minimal surface tension of surfactant alone. Surfactant activity was evaluated *in vivo* by its capacity to restore gas exchange in a standardized model for acute respiratory insufficiency in adult rats.

Methods

Surfactant and antibiotics

A freeze-dried natural surfactant was used, isolated from bovine lungs as previously described (Gommers *et al.*, 1993). It consists of approximately 90–95% phospholipids, 1% hydrophobic proteins (surfactant-proteins B and C) and 1% free fatty acids, the remainder being other lipids such as cholesterol and glyceride; there was no surfactant-protein A in this surfactant preparation.

The commercial formulations of the antibiotics for intravenous administration were used in all experiments: amphotericin B (Bristol-Myers Squibb, Woerden, The Netherlands), amoxicillin (SmithKline Beecham, Rijswijk, The Netherlands), ceftazidime (Glaxo, Nieuwegein, The Netherlands), pentamidine (Rhône-Poulenc Rorer, Amstelveen, The Netherlands), tobramycin (Eli Lilly, Nieuwegein, The Netherlands). The dosages used in these experiments were based upon maximal daily dosages for adults: amphotericin B 1.5 mg kg^{-1}, amoxicillin 200 mg kg^{-1}, ceftazidime 150 mg kg^{-1}, pentamidine 4 mg kg^{-1} and tobramycin 10 mg kg^{-1}. Surfactant and antibiotic-surfactant suspensions were freshly made for each experiment. The surfactant powder was suspended in 0.9% NaCl solution, except in group 8 (see below) where 0.2 M $NaHCO_3$ was used as solvent. Antibiotics were dissolved in 0.9% NaCl or in H_2O (amphotericin B). The antibiotic solutions were added to the surfactant suspension and handshaken.

Minimal surface tension measurements

Minimal surface tension of each antibiotic with and without additional surfactant was measured and compared with the minimal surface tension of surfactant alone. Samples of surfactant, antibiotic and antibiotic-surfactant mixtures were freshly made in duplicate. A low surfactant concentration (1 mg total lipids ml^{-1} saline) was used to facilitate the detection of changes in the minimal surface tension when antibiotics were added to this surfactant solution. The antibiotic concentrations of the samples were similar to the antibiotic concentrations used in the *in vivo* experiments (Table 1).

Minimal surface tensions of the samples were measured using a modified Wilhelmy balance (E. Biegler GmbH, Mauerbach, Austria) which keeps the temperature constant at 37°C. The trough was filled with warm saline (37°C) and calibrated. After calibration, 50 μl of a sample (containing 50 μg total lipids) was placed upon the surface, by use of an eppendorf pipette. Two minutes were waited for spreading of the sample. Subsequently, the measurement was continued. Surface area was compressed and expanded with a cycling time of 3 min per cycle and maximum and minimum surface areas of 64 and 12.8 cm^2, respectively (100% and 20%). Minimal surface tension was measured after 3 cycles at 20% surface area, and is expressed as milli Newton $metre^{-1}$ (mN m^{-1}) (Notter, 1984).

Table 1 Mean minimal surface tension of the antibiotics with and without surfactant

	Concentration (mg ml^{-1})	*Minimal surface tension* (mN m^{1})	*with surfactant*
Saline	–	72.8±0.3	20.9±0.4
Amphotericin B	0.375	53.4±0.4	21.4±0.6
Amoxicillin	50	65.3±3.1	20.6±0.8
Ceftazidime	37.5	61.2±3.9	22.1±0
Pentamidine	1	59.2±2.4	18.1±0.8
Tobramycin	2.5	68.1±0.8	21.4±0.4

Values are means±s.d.

Animal studies

The study protocol was approved by the institutional Animal Care Committee. Male Sprague-Dawley rats (SPF, Iffa Credo, Belgium), mean bodyweight 275±20 g, were used in all experiments.

Respiratory failure was induced by lung lavage as described previously (Lachmann *et al.*, 1980). Briefly; under inhalation anaesthesia, O_2, N_2O and Isoflurane 2% (65:33:2), the trachea and the carotid artery were cannulated. Rats were connected to the ventilator. Anaesthesia and muscle relaxation was maintained during the experiment with pentobarbitone sodium (60 mg kg^{-1} intraperitoneally) and pancuronium bromide (0.5 mg kg^{-1}, intramuscularly) every hour. Lungs were lavaged 5–7 times with 30 ml kg^{-1} bodyweight of warm saline to achieve a PaO_2 <80 mmHg at the following ventilator settings using a Servo Ventilator 300 (Siemens-Elema, Solna, Sweden): pressure-controlled ventilation, frequency = 30 breaths min^{-1}, peak airway pressure = 26 cmH_2O, positive end expiratory pressure (PEEP) = 6 cmH_2O, I:E ratio = 1:2 and FiO_2 = 1. These ventilation settings were maintained throughout the study period.

There were 9 different treated groups. Volume instilled intratracheally was 4 ml kg^{-1} bodyweight (BW).

(1) – n = 17, surfactant, 160 mg kg^{-1} BW (40 mg ml^{-1}); (2) – n = 6, air; (3) – n = 6, saline; (4) – n = 6, surfactant + amphotericin B, 1.5 mg kg^{-1} BW (0.375 mg ml^{-1}); (5) – n = 6, surfactant + amoxicillin, 200 mg kg^{-1} BW (50 mg ml^{-1}); (6) – n = 6, surfactant + ceftazidime, 150 mg kg^{-1} BW (37.5 mg ml^{-1}); (7) – n = 6, surfactant + pentamidine, 4 mg kg^{-1} BW (1 mg ml^{-1}); (8) – n = 6, surfactant + tobramycin, 10 mg kg^{-1} BW in saline (2.5 mg ml^{-1}); (9) – n = 6, surfactant + tobramycin, 10 mg kg^{-1} BW in $NaHCO_3$ (2.5 mg ml^{-1}).

Treatment with surfactant, air, saline or an antibiotic-surfactant mixture was started within 6–10 min after the last lavage. For this, rats were disconnected from the ventilator and the 4 ml kg^{-1} bolus of surfactant, air, saline or antibiotic-surfactant mixture was instilled intratracheally followed by insufflation of 24 ml kg^{-1} of air. After instillation, animals were immediately reconnected to the ventilator. Blood samples were taken from the carotid artery of each rat shortly before and 5 min after the lung lavage procedure (t = 0 min) and then at t = 5, 30, 60, 90 and 120 min post treatment. Blood gas values were measured with the ABL 505 Acid-Base Laboratory (Radiometer, Copenhagen, Denmark).

Each experiment consisted of six rats placed at one ventilatory unit. In each experiment one or two positive surfactant controls were included. The surfactant group consisted, therefore, of 17 animals. All other treatment groups consisted of 6 rats per group. At the end of the observation period animals were killed by an intraperitoneal overdose of pentobarbitone.

Statistical analysis

Data are expressed as the mean±standard deviation (s.d.). In the *in vivo* study statistical significant differences were evaluated with an analysis of variance (ANOVA) for repeated measurements by use of the GLM procedure of the SAS statistical package (SAS, 1990). Tests performed were: (1) within group, the effect of time on changes in PaO_2 and $PaCO_2$ and (2)

the difference in PaO_2 and $PaCO_2$ values between groups, using the surfactant-treated group as a positive control and the saline and air treated groups as negative controls. Tests were performed from $t=0$ min to $t=120$ min to evaluate overall differences between and within groups. To evaluate the acute effect of surfactant treatment (within 5 min), tests were performed from $t=0$ min to $t=5$ min and to evaluate the stability of PaO_2 increases tests were performed from $t=30$ min to $t=120$ min. Statistical significance was accepted at a P value $\leqslant 0.05$.

Results

In vitro

Table 1 shows the minimal surface tension (mean ± s.d., $n=2$) of the samples. Addition of 1 mg ml^{-1} surfactant to the antibiotic solution decreased minimal surface tension to levels comparable to that of surfactant alone. When surfactant alone was applied to the surface in higher concentrations ($\geqslant 3$ mg ml^{-1}) the minimal surface tension would decrease further to values below 5 mN m^{-1}.

In vivo

When comparing the antibiotic-surfactant treated groups with the surfactant treated group significant differences in blood gas values over time were found when surfactant was mixed with amphotericin B, amoxicillin or tobramycin. PaO_2 and $PaCO_2$ levels in the ceftazidime-surfactant treated group and the pentamidine-surfactant treated group were not significantly different at any time point from those in the surfactant treated group (Figure 1a–d and Table 2).

In the amphotericin B-surfactant treated group, PaO_2 levels initially increased comparable to the PaO_2 increase in the surfactant treated group. However, in time PaO_2 levels decreased significantly compared to the surfactant treated group (Figure 1a). $PaCO_2$ levels were increased compared to the surfactant treated group (Table 2).

In the amoxicillin-surfactant treated group the initial rise in PaO_2 (at 5 min) as well as the PaO_2 levels in the subsequent 120 min were significantly decreased compared to PaO_2 levels in the surfactant treated group (Figure 1b). In time PaO_2 levels tended to rise in the amoxicillin-surfactant treated group; however, this increase was not statistically significant ($P=0.055$, within subjects $t5$ min – $t120$ min). $PaCO_2$ levels were significantly increased from 30–120 min compared to the surfactant treated group.

After instillation of tobramycin-surfactant suspended in saline, PaO_2 levels varied between the animals and were on average lower than PaO_2 after surfactant instillation (Figure 1d). PaO_2 levels tended to decrease in time ($P=0.054$, within subjects effect $t5$ min – $t120$ min). $PaCO_2$ levels were significantly higher in the tobramycin-surfactant group when compared to the surfactant treated group (Table 2).

Preparing the solutions for the *in vivo* tests showed that addition of tobramycin to surfactant suspended in saline resulted in a precipitation of the suspension. Since aminoglycosides are known to bind to negatively charged phospholipids (Mingeot-Leclercq *et al.*, 1988) the effect of the pH of the solution on visible precipitation was studied. It was found that at a pH of 8.3 when using 0.2 M $NaHCO_3$ as solvent, no visible precipitation occurred when tobramycin was added to the surfactant suspension.

PaO_2 levels in the group receiving tobramycin-surfactant suspended in 0.2 M $NaHCO_3$ were uniform and not significantly different from PaO_2 levels in the surfactant treated group (Figure 1d).

In all groups receiving either surfactant or an antibiotic-surfactant mixture PaO_2 levels were significantly higher than PaO_2 levels in the air or saline treated group (Figure 1a–d).

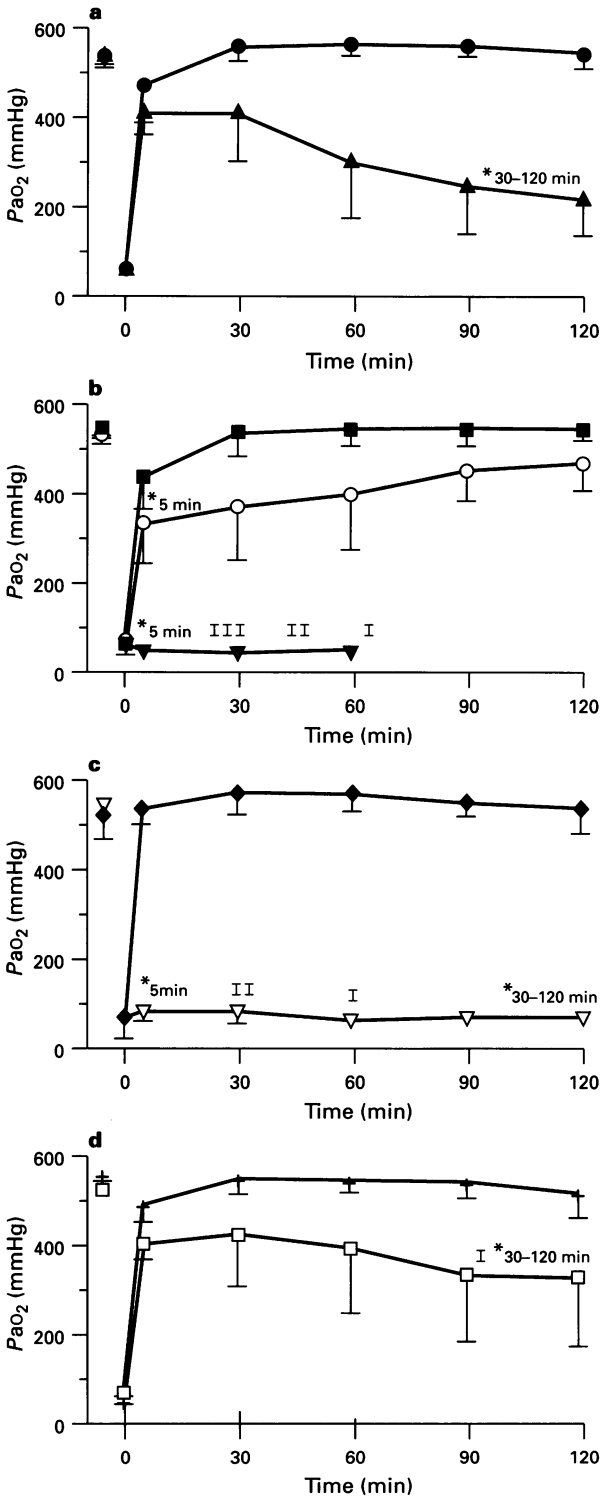

Figure 1 PaO_2 values (mean ± s.d.) over time for the nine treated groups. (a) Surfactant marked (●) and amphotericin B-surfactant marked (▲); (b) saline marked (▼), amoxicillin-surfactant marked (○) and ceftazidime-surfactant marked (■); (c) pentamidine-surfactant marked (◆) and air marked (▽); (d) tobramycin-surfactant in saline marked (□) and tobramycin-surfactant in $NaHCO_3$ marked (+). Animals who died during the study period are marked with an I. $*_{5\,min}$ $P<0.05$, for differences between groups over $t=0$ min – $t=5$ min when compared to the surfactant treated group (ANOVA, repeated time measurements). $*_{30-120\,min}$ $P<0.05$, for differences between groups over $t=30$ min – $t=120$ min when compared to the surfactant treated group (ANOVA, repeated time measurements).

Table 2 Mean $PaCO_2$ values (mmHg) of the nine different treated groups

	Pre-lavage	*t = 0 min*	*t = 5 min*	*t = 30 min*	*t = 60 min*	*t = 90 min*	*t = 120 min*	P *values (compared to surfactant group)* *t0 min-t120 min*	*t0 min-t5 min*	*t30 min-t120 min*
Surfactant	40±6.1	73±11	46±9.2	42±8.2	39±8	39±6.7	37±7			
Surfactant-amphotericin B	34±2.2	79±7	54±6.1	45±10.9	44±8.5	49±8.8	58±14.8	0.056	0.198	0.044
Surfactant-amoxicillin	36±2.5	71±7.1	52±5.8	51±9.8	49±10.2	46±7.7	47±9	0.082	0.645	0.026
Surfactant-ceftazidime	34±5.1	74±7.1	47±8.8	42±9.3	41±8.8	39±7.4	36±9.2	0.940	0.856	0.996
Surfactant-pentamidine	38±6.5	72±5.7	45±9.4	42±9.1	41±13.4	40±10.5	41±9.7	0.840	0.784	0.653
Surfactant-tobramycin in saline	36±3.4	78±8.9	55±6.9	49±7.3	49±9.7	50±9.3	51*±9.2	0.040	0.112	0.020 0.191
Surfactant-tobramycin in 0.2M $NaHCO_3$	35±1.2	70±9.4	50±6.1	45±6.9	43±7.2	42±5.5	41±7.1	0.363	0.912	0.001
Air	36±10.4	67±17.3	53±16.7	48**±17.3	47***±17.1	47***±19.7	46***±18.3	0.001	0.255	
Saline	37±3.4	73±15.5	89±18.7	132***±22.9	155*****				0.001	

Values shown are mean±s.d. *P values for between group differences in the $PaCO_2$ values of the surfactant treated group vs the surfactant-antibiotic treated group, determined with ANOVA for repeated time measurements. *Indicates the number of animals that died during the experiment.

Discussion

This study investigated the influence of five antimicrobial agents on pulmonary surfactant function to assess the possible use of surfactant as a pulmonal drug delivery system. It was found that surfactant function was unaffected when mixed with ceftazidime and pentamidine. Surfactant function was reduced when combined with amphotericin B and amoxicillin. With tobramycin-surfactant mixtures, surfactant activity was reduced when saline was used as solvent. Surfactant function was, however, unaffected in the presence of tobramycin when 0.2 M $NaHCO_3$ was used as solvent.

It has been previously discussed that evaluation of surfactant function *in vitro* is valuable. The *in vitro* results will, however, not accurately predict surfactant function *in vivo* (Lachmann, 1986; Jobe & Ikegami, 1987). In the present study *in vitro* examination was, therefore, limited to the question whether minimal surface tension of the antibiotic-surfactant mixtures was comparable to the minimal surface tension of surfactant alone. The minimal surface tensions did not vary strongly between surfactant and antibiotic-surfactant mixtures which encouraged us to evaluate the mixtures *in vivo*.

In vivo surfactant function was evaluated in a standardized model of surfactant deficiency in adult animals induced by whole lung lavage with warm saline. This model has been used extensively for testing various aspects of exogenous surfactant therapy (Lachmann *et al.*, 1983; Kobayashi *et al.*, 1984; Lachmann, 1986; Gommers *et al.*, 1993; Lewis *et al.*, 1993; Häfner *et al.*, 1995). One of the advantages of this model is that the level of induced lung damage can be excellently standardized (Lachmann & van Daal, 1992).

Two properties of the pulmonary surfactant can be evaluated in this model. First, its capacity to open up the atelectatic lung which is characterized by an immediate increase in PaO_2. Second its capacity to keep the lung open over a longer period without changing the ventilatory settings, which is characterized by an unchanged PaO_2 over time (Gommers *et al.*, 1993). When exogenous surfactant is merely used for delivery of agents in the peripheral regions, one can assume that the first quality (to open up the lung) is most important. However, when surfactant-antibiotic mixtures are simultaneously used for treatment of respiratory failure, the second quality (to keep the lung open) is essential for proper surfactant therapy. Therefore, we evaluated the results from the first five minutes after instillation and the results from 30 to 120 min separately.

Instillation of amphotericin B-surfactant mixtures improved gas exchange within five minutes. In time, however, the gas exchange deteriorated, which indicates an inhibition of the surfactant function. To our knowledge, interactions between surfactant function and amphotericin B have not been reported before and the results should be interpreted with care. Studies in patients receiving amphotericin B delivered as an aerosol or instilled endotracheally have reported minimal or no side effects (Beyer *et al.*, 1994). A study on aerosolized amphotericin B in rats showed that it was well tolerated and produced no histopathologic changes in the lungs (Niki *et al.*, 1990). Although rare, lung injury has been found when amphotericin B was instilled intravenously (Levine *et al.*, 1991; Hardie *et al.*, 1992).

In the present study a high dose of amphotericin B was instilled directly into a severely damaged lung. Plausible mechanisms involved in the observed inhibition of surfactant by amphotericin B could be direct interaction of the agent with surfactant or an interaction of the agent with the alveolar capillary membrane resulting in an influx of plasma proteins. Plasma proteins are well-known inhibitors of pulmonary surfactant function (Holm, 1992).

The initial increase in gas exchange after instillation of the amoxicillin-surfactant mixture was decreased compared to the surfactant treated group but gradually improved with time. As with amphotericin B, the cause for the changed surfactant function is unknown. One explanation could be that amox-

icillin binds to the specific surfactant proteins B and C present in this surfactant preparation. Both Sp-B and Sp-C are an important factor in the physical surfactant function (for review see Johansson *et al.*, 1994).

A previous study from our group showed that tobramycin activity was decreased in the presence of surfactant (van 't Veen *et al.*, 1995). Binding of aminoglycosides to negatively-charged phospholipids has been described as a mechanism for the nephrotoxic action of these antibiotics (Mingeot-Leclercq *et al.*, 1988). Therefore, it was speculated that the decreased activity of tobramycin found in the presence of surfactant was induced by tobramycin binding to phospholipids in the surfactant.

The present study showed that when surfactant and tobramycin are dissolved in saline (pH = 6.3) a precipitation occurred. Instillation of this mixture in lavaged lungs resulted in a decreased surfactant function. Since the charge of the phospholipids and/or the tobramycin seemed to be relevant, the effect of the pH of the solvent was investigated. When tobramycin and surfactant were suspended in 0.2 M $NaHCO_3$ (pH = 8.3) the suspension was homogeneous at sight. Restoration of gas exchange after instillation of this mixture in lavaged rats was uniform and not different from that in rats treated with surfactant only.

The purpose of this study was to investigate possible interactions between antimicrobial agents and an exogenous pulmonary surfactant. This study together with a previous study from our group (van 't Veen *et al.*, 1995) demonstrated that interactions between antimicrobial agents and exogenous surfactant exist and may influence the activity of both substances.

Due to the differences in chemical composition between all currently available surfactant preparations (Fujiwara & Robertson, 1992) extrapolation of the present results to other surfactant preparations is not recommended. It has been shown in several studies that the compositional differences have a large impact on the *in vitro* and *in vivo* physical behaviour of the surfactant preparations (Ikegami *et al.*, 1987; Cummings *et al.*, 1992; Fujiwara & Robertson, 1992; Häfner *et al.*, 1995). Accordingly it can be expected that possible interactions between exogenous surfactant and other agents differ between the various surfactant preparations. Therefore, before surfactant-antibiotic mixtures are used in clinical trials, alterations in activity of both substances should be considered and carefully examined.

This study further showed that simple changes, such as the use of a different solution for suspending the surfactant, can overcome changes in surfactant activity due to interactions between surfactant and antibiotics. Therefore, although the results with amphotericin B are poor in this study this should not definitely exclude the use of amphotericin B-surfactant mixtures. For example, as with tobramycin, the use of other solvents could be investigated.

The use of surfactant as a delivering agent for antibiotics is expected to have great potential in selected patient groups. However, questions remain open on both distribution patterns in infected lungs and *in vivo* efficacy. Future studies should, therefore, focus on these issues.

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