Influence of Pulmonary Surfactant on In Vitro Bactericidal Activities of Amoxicillin, Ceftazidime, and Tobramycin

ANNEMARIE VAN 'T VEEN,[1] JOHAN W. MOUTON,[2] DIEDERIK GOMMERS,[1]
JAN A. J. W. KLUYTMANS,[2] PAUL DEKKERS,[1]
AND BURKHARD LACHMANN[1]*

*Departments of Anaesthesiology[1] and Clinical Microbiology,[2] Erasmus University Rotterdam,
Rotterdam, The Netherlands*

The influence of a natural pulmonary surfactant on antibiotic activity was investigated to assess the possible use of exogenous surfactant as a vehicle for antibiotic delivery to the lung. The influence of surfactant on the bactericidal activity of amoxicillin was tested against *Staphylococcus aureus* and *Streptococcus pneumoniae*, and the influence of surfactant on the activities of ceftazidime and tobramycin was tested against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *S. aureus*, and *S. pneumoniae*. In vitro antibiotic activity was determined by killing curve studies in media with and without surfactant. Amoxicillin and ceftazidime activities were not changed in the presence of surfactant, except for a decreased killing rate of *S. pneumoniae* by ceftazidime in medium with additional rabbit serum. In contrast, killing curves with low concentrations of tobramycin ($0.25\times$ and $1\times$ the MIC) showed a decreased level of activity of tobramycin against all pathogens tested in the presence of surfactant. With higher tobramycin concentrations ($4\times$ the MIC) killing rates were decreased less or were unchanged in the presence of surfactant. Concluding from the results of the study, both amoxicillin and ceftazidime can be combined with surfactant without the loss of activity. For mixing surfactant with tobramycin, dosages should be adjusted to overcome the partial inactivation of tobramycin by surfactant.

Lower respiratory tract infections remain an important cause of morbidity and mortality, despite the use of new potent antibiotics. Nosocomial pneumonia remains an infection that is especially difficult to treat, with crude mortality rates of 10 to 30% and even higher rates in specific patient groups (9, 43). Efficient antimicrobial therapy is considered to be dependent on appropriate antibiotic concentrations at the site of infection (1, 43). For pneumonia this is within the alveolar space, together with the epithelial lining fluid and the lung interstitium (40). However, the high systemic doses of some antibiotics needed to reach therapeutic levels at these sites may be accompanied by adverse side effects, e.g., oto- and nephrotoxicities caused by aminoglycosides (17, 42).

Local administration of antibiotics via the trachea offers the potential benefits of delivering high antibiotic concentrations to the site of infection and low systemic absorption. Previous studies have shown beneficial effects from both the inhalation of antibiotics via aerosol (14, 32, 35) and the direct endotracheal instillation of antibiotics (4, 5, 22). However, with aerosol inhalation the amount of antibiotics deposited in the lung is small (only 10 to 20%), even with the best nebulizers (4, 16, 25). Moreover, pulmonary deposition is particularly high in the central airways and decreases toward the periphery in patients with decreased pulmonary function (16, 24). With direct endotracheal instillation, distribution is also largely limited to the central airways (3). Thus, the therapeutic efficacies of these modes of administration are limited, especially since the location of infected areas is most often peripheral. The efficacies of locally administered antibiotics might therefore be improved by optimizing the distribution within the lung.

Because of the small diameters of the airways in the periph-

ery of the lung, fluids with a high surface tension, such as saline, require high pressures for passage through these airways (27). Surfactant, a mixture of phospholipids and specific surfactant proteins, has the capacity to lower surface tension (44). Recently, it has been shown that use of an exogenous pulmonary surfactant labelled with a radioactive colloid and mixed with pentamidine results in a more peripheral and uniform distribution pattern in the lungs compared with that obtained with a combination of pentamidine and saline (21). It is therefore expected that the use of surfactant as a carrier for antibiotics has great potential in treating patients with severe pneumonia.

However, it is unknown if surfactant affects the activities of antibiotics. Therefore, an in vitro study was designed to investigate the influence of a bovine surfactant on the bactericidal activities of clinically relevant antibiotics against pathogens often involved in respiratory tract infections.

MATERIALS AND METHODS

Bacteria and preparation of inoculum. The following strains were used: *Klebsiella pneumoniae* ATCC 43816, *Staphylococcus aureus* ATCC 25923, *Streptococcus pneumoniae* ATCC 6301, and *Pseudomonas aeruginosa* ATCC 27853. For *S. aureus* and *K. pneumoniae*, stationary-phase cultures were prepared by incubation for 16 h at 37°C in Mueller-Hinton broth (MHB; Difco Laboratories, Detroit, Mich.). For *P. aeruginosa*, MHB was supplemented with magnesium (12.5 mg/liter) and calcium (20 to 25 mg/liter) (Merck, Darmstadt, Germany) (33). After proper dilution and reincubation for 2 h at 37°C, suspensions of logarithmically growing bacteria were obtained. For an *S. pneumoniae* inoculum, an overnight culture on a 5% blood agar plate (Bactim, Breukelen, The Netherlands) was suspended in Todd-Hewitt broth (THB; Oxoid Ltd., Basingstoke, England) containing 10% normal rabbit serum (Dako A/S, Glostrup, Denmark), which was first inactivated for 30 min at 56°C. The culture was then incubated at 37°C, and the optical density was measured repeatedly. When the optical density remained constant for 30 min, the culture was diluted with THB to obtain the proper inoculum size of end-log-phase bacteria.

Antibiotics. Tobramycin (Eli Lilly, Amsterdam, The Netherlands), amoxicillin (SmithKline Beecham, Amstelveen, The Netherlands), and ceftazidime (Glaxo Pharmaceuticals Ltd., Greenford, England) were kindly provided by the manufacturers and were prepared by diluting the standard powder or solution with the

* Corresponding author. Mailing address: Department of Anaesthesiology, Room EE 2393, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands. Phone: 31 10 4087312. Fax: 31 10 4367870.

recommended diluent to a stock solution of 2,560 µg/ml, which was stored at −80°C in small aliquots. For each experiment a fresh aliquot was used and was diluted in broth to obtain the appropriate concentrations.

Surfactant. The surfactant used in the studies was a freeze-dried natural surfactant isolated from bovine lungs as described previously (13). It consisted of approximately 90 to 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. For every killing curve study or bacterial growth study, the surfactant was suspended in broth to a concentration of 25 mg/ml of growth medium.

Antimicrobial susceptibility tests. The MIC was determined by a microdilution technique. The MIC of the drug was defined as the lowest concentration that suppressed visible growth after incubation of 5×10^5 CFU/ml for 18 h at 37°C in microtiter plates containing a total volume of 200 µl. For *K. pneumoniae* and *S. aureus* this was determined in MHB, for *P. aeruginosa* this was determined in MHB supplemented with Mg^{2+} and Ca^{2+}, and for *S. pneumoniae* this was determined in THB. MICs did not differ by more than one step from the MICs for these strains reported earlier (28, 36).

Time-kill curves were made at 0.25×, 1×, and 4× the MIC of the tested antibiotic. Killing curves were calculated from growth in 10-ml glass tubes containing a total volume of 2 ml of growth medium, with or without 25 mg of surfactant per ml. For *S. pneumoniae*, the influence of surfactant on bacterial killing by antibiotics was determined in THB with and without 10% rabbit serum. With each assay appropriate growth controls were studied. The starting inoculum (at time zero) was adjusted to approximately 5×10^5 CFU/ml. Sampling was performed at 0, 1, 2, 4, 6, and 8 h after inoculation. Tenfold dilution steps of 100-µl samples were made in phosphate-buffered saline (pH 7.3; Oxoid Ltd., Basingstoke, England) on ice. In order to determine the number of viable microorganisms, 100 µl of the appropriate 10-fold dilution steps was plated on Iso-Sensitest agar (Oxoid Ltd.) or, in the case of *S. pneumoniae*, on blood agar plates. Colony counts were determined after 24 h of incubation at 37°C. To minimize antibiotic carryover on subculture plates, only 50 µl of an undiluted sample was subcultured directly onto the agar plate. The lower limit of detection by this method was 20 CFU/ml, and the number that could be accurately counted was 600 CFU/ml. Killing curve studies were performed at least in duplicate.

Other measurements. The pH of the incubation medium was measured to assess a possible change in pH when surfactant and/or rabbit serum was added to the broth. For this, volumes of 2 ml of broth with or without 25 mg of surfactant per ml and with or without 10% rabbit serum were freshly made in duplicate. The pHs of these samples were measured with a glass electrode (model 920 A; Orion Research Inc., Boston, Mass.).

The nonbound tobramycin concentrations in samples with and without surfactant and with or without rabbit serum in THB were determined in duplicate by using the Amicon microparition system (MPS-1, 4010; Amicon, Danvers, Mass.). For this, 1-ml samples were made. These samples contained approximately 100 µg of tobramycin and the following: (i) no extra additions (controls), (ii) 25 mg of surfactant per ml, (iii) 12.5 mg of surfactant per ml, and (iv) 10% rabbit serum. The samples were centrifuged at $2,000 \times g$ for 30 min. Tobramycin concentrations were determined for filtration in the sample and after filtration in the filtrate by fluorescence analysis with the TDX system (Abbott Diagnostic Division, Weesp, The Netherlands). Binding is expressed as [1 − (ratio of free tobramycin/total tobramycin)] · 100%.

Statistical analysis. For analysis of the data, a logarithmic transformation ($\log_{10}$) was performed on all data. With killing curves, the total area under the killing curve of transformed data was determined for presentation of the data. In this type of analysis the rate of killing is inversely related to the area under the killing curve (41). The effect of surfactant on antibiotic activity (with surfactant versus without surfactant) was tested by analysis of variance for repeated measures by using the GLM procedure of the SAS statistical package (39). With these tests the overall effect of surfactant on antibiotic activity as well as the effect of surfactant for each antibiotic concentration separately were tested. Additionally, for *S. pneumoniae* the effect of rabbit serum was tested.

Growth rate was expressed as the slope (y/x) of the regression line from time zero to 8 h, and the units of growth rate were the change in the $\log_{10}$ CFU per milliliter per hour (28). The means and standard deviations were calculated. Statistical significance between the growth rate of bacteria in medium with surfactant versus that in medium without surfactant was tested by the *t* test for two samples (39). With all statistical tests, significance was accepted at *P* values of ≤0.05 (two-tailed).

RESULTS

Bacterial growth rate ($\log_{10}$ CFU per milliliter per hour) was not altered for *P. aeruginosa*, *K. pneumoniae*, or *S. aureus* in the presence of surfactant (Table 1). For *S. pneumoniae*, the bacterial growth rate was not affected except for a decreased growth rate in THB with rabbit serum in the presence of surfactant (Table 1). No statistically significant difference was found between the growth rate of *S. pneumoniae* in THB with rabbit serum ($0.509 \pm 0.03 \log_{10}$ CFU/ml/h) and the growth

rate in THB with rabbit serum and surfactant ($0.437 \pm 0.03 \log_{10}$ CFU/ml/h) compared with the growth rate in THB alone ($P = 0.064$ and 0.606, respectively). Similarly, no statistically significant difference was found between the growth rate of *S. pneumoniae* in THB with rabbit serum ($0.509 \pm 0.03 \log_{10}$ CFU/ml/h) and the growth rate in THB with rabbit serum and surfactant ($0.437 \pm 0.03 \log_{10}$ CFU/ml/h) compared with the growth rate in THB with surfactant and without serum ($0.481 \pm 0.007 \log_{10}$ CFU/ml/h) ($P = 0.060$ and 0.306, respectively).

Bacterial killing of *S. pneumoniae* and *S. aureus* by amoxicillin and of *K. pneumoniae*, *P. aeruginosa*, and *S. aureus* by ceftazidime was not altered in the presence of surfactant (Tables 2 and 3). The killing of *S. pneumoniae* by ceftazidime in THB supplemented with rabbit serum was decreased in the presence of surfactant at 4× the MIC (Table 3). A reduction in the bactericidal activity (increased areas under the killing curve) of tobramycin against *K. pneumoniae*, *P. aeruginosa*, *S. aureus*, and *S. pneumoniae* was observed when surfactant was added to the medium (Table 4). This was best demonstrated in killing curves at the MIC for the tested strain. The effect of surfactant on bacterial killing by tobramycin was less (*S. aureus*

TABLE 1. Bacterial growth rates

Organism	Bacterial growth rate ($\log_{10}$ CFU/ml/h [mean ± SD])		P value[a]
	Without surfactant	With surfactant	
K. pneumoniae	0.524 ± 0.084	0.576 ± 0.053	0.414
P. aeruginosa	0.349 ± 0.018	0.344 ± 0.010	0.652
S. aureus	0.474 ± 0.048	0.463 ± 0.046	0.740
S. pneumoniae			
Without serum	0.403 ± 0.076	0.481 ± 0.007	0.131
With serum	0.509 ± 0.025	0.437 ± 0.033	0.015

[a] The *P* value for bacterial growth in medium with surfactant versus that for bacterial growth in medium without surfactant was determined by the *t* test.

TABLE 2. Influence of surfactant on amoxicillin activity

Organism	Amoxicillin concn (µg/ml)	Area under killing curve [($\log_{10}$ CFU/ml) × h (mean ± SD)]		P value	
		Without surfactant	With surfactant	Be-tween[a]	Over-all[b]
S. aureus	0.03	47.99 ± 4.46	43.40 ± 0.17	0.303	0.774
	0.13	29.42 ± 1.15	29.29 ± 1.12	0.917	
	0.5	22.88 ± 0.17	22.40 ± 5.05	0.861	
S. pneumoniae					
Without serum	0.03	52.32 ± 1.80	55.11 ± 0.38	0.174	0.393
	0.13	29.94 ± 0.26	17.28 ± 5.76	0.061	
	0.5	19.77 ± 4.53	15.51 ± 4.75	0.521	
With serum	0.03	53.57 ± 0.47	55.72 ± 0.36	0.346	0.394
	0.13	28.84 ± 1.98	26.70 ± 1.12	0.717	
	0.5	19.43 ± 4.33	17.39 ± 6.40	0.489	

[a] The *P* values for the effects of surfactant on antibiotic activity for each antibiotic concentration were determined by analysis of variance for repeated measures.
[b] The *P* values for the effects of surfactant on antibiotic activity in all killing curves were determined by analysis of variance for repeated measures.

TABLE 3. Influence of surfactant on ceftazidime activity

Organism	Ceftazidime concn (μg/ml)	Area under killing curve [(log$_{10}$ CFU/ml) × h (mean ± SD)]		P value	
		Without surfactant	With surfactant	Be-tween[a]	Over-all[b]
K. pneumoniae	0.13	19.90 ± 0.12	25.17 ± 3.08	0.346	0.378
	0.5	16.32 ± 2.62	17.88 ± 2.42	0.717	
	2	8.30 ± 8.20	13.11 ± 3.85	0.489	
P. aeruginosa	0.5	54.38 ± 2.25	51.70 ± 5.69	0.337	0.799
	2	38.59 ± 0.59	38.52 ± 0.10	0.409	
	8	35.23 ± 3.08	32.54 ± 0.96	0.348	
S. aureus	0.03	49.77 ± 0.75	43.78 ± 3.71	0.184	0.602
	0.13	27.74 ± 3.13	32.25 ± 0.39	0.250	
	0.5	24.84 ± 3.38	30.67 ± 0.50	0.158	
S. pneumoniae					
Without serum	0.03	48.79 ± 2.46	52.54 ± 0.90	0.100	0.570
	0.13	24.51 ± 4.23	15.43 ± 3.51	0.076	
	0.5	16.48 ± 0.69	15.15 ± 0.90	0.198	
With serum	0.03	51.92 ± 1.49	52.99 ± 0.37	0.329	0.003
	0.13	22.32 ± 5.74	27.90 ± 1.41	0.120	
	0.5	18.41 ± 3.53	26.88 ± 1.24	0.050	

[a] The P values for the effects of surfactant on antibiotic activity for each antibiotic concentration were determined by analysis of variance for repeated measures.
[b] The P values for the effects of surfactant on antibiotic activity in all killing curves were determined by analysis of variance for repeated measures.

TABLE 4. Influence of surfactant on tobramycin activity

Organism	Tobramycin concn (μg/ml)	Area under killing curve [(log$_{10}$ CFU/ml) × h (mean ± SD)]		P value	
		Without surfactant	With surfactant	Be-tween[a]	Over-all[b]
K. pneumoniae	0.13	64.48 ± 0.80	64.94 ± 0.01	0.222	0.001
	0.5	13.66 ± 0.27	61.92 ± 0.31	0.000	
	2	3.54 ± 0.92	4.04 ± 1.63	0.742	
P. aeruginosa	0.5	25.71 ± 4.53	41.22 ± 2.45	0.038	0.567
	2	5.34 ± 3.46	2.89 ± 0.00	0.423	
	8	2.89 ± 0.00	2.89 ± 0.00	1.000	
S. aureus	0.03	51.67 ± 4.18	53.13 ± 0.99	0.727	0.016
	0.13	12.56 ± 10.64	39.41 ± 15.17	0.169	
	0.5	6.15 ± 1.22	20.81 ± 1.57	0.011	
S. pneumoniae					
Without serum	4	48.67 ± 5.03	55.74 ± 0.54	0.206	0.001
	16	15.23 ± 2.67	54.26 ± 0.18	0.002	
	64	4.35 ± 0.86	32.73 ± 1.19	0.001	
With serum	4	55.96 ± 2.17	54.86 ± 0.38	0.610	0.005
	16	54.63 ± 1.83	54.49 ± 0.14	0.651	
	64	21.90 ± 6.46	39.24 ± 0.27	0.001	

[a] The P values for the effects of surfactant on antibiotic activity for each antibiotic concentration were determined by analysis of variance for repeated measures.
[b] The P values for the effects of surfactant on antibiotic activity in all killing curves were determined by analysis of variance for repeated measures.

and *S. pneumoniae*) or disappeared (*K. pneumoniae* and *P. aeruginosa*) when tobramycin concentrations were increased to 4× the MIC for the tested pathogen (Table 4). Typical examples of decreased tobramycin activity in the presence of surfactant against *S. pneumoniae* and *K. pneumoniae* are shown in Fig. 1 and 2, respectively. The addition of rabbit serum to THB did not alter the overall killing rates of *S. pneumoniae* by amoxicillin ($P = 0.647$) or ceftazidime ($P = 0.201$) compared with the bacterial killing rate in THB alone. Overall killing by tobramycin in medium with rabbit serum was decreased compared with killing in THB alone ($P < 0.001$).

The pH of the incubation medium was not affected by the addition of surfactant and/or rabbit serum to the broth. The change in pH was <0.05 in all experiments. Tobramycin binding was (i) 13% in THB alone (controls), (ii) 30% in THB plus 25 mg of surfactant per ml, (iii) 19% in THB plus 12.5 mg of surfactant per ml, and (iv) 21% in THB plus rabbit serum. Differences between duplicate determinations were <2% in all experiments.

DISCUSSION

In the present study, the influence of a natural pulmonary surfactant on antibiotic activity was investigated to assess the possible use of surfactant as a vehicle for delivering antibiotics to the lung. The results show that both amoxicillin and ceftazidime can be combined with pulmonary surfactant without the loss of antibiotic activity. Tobramycin activity was reduced in the presence of surfactant, but this could be overcome by increasing the tobramycin concentration. Furthermore, it can be concluded that pulmonary surfactant can alter antibiotic

activity; therefore, antibiotic activity should be tested before using surfactant-antibiotic mixtures for the treatment of severe pneumonia.

Several studies have reported the inactivation of aminoglycosides, as was found with tobramycin in the present study. The pH strikingly alters the bioactivities of aminoglycosides. The MIC increases, depending on the organism and the particular aminoglycoside involved, when the pH of the culture medium
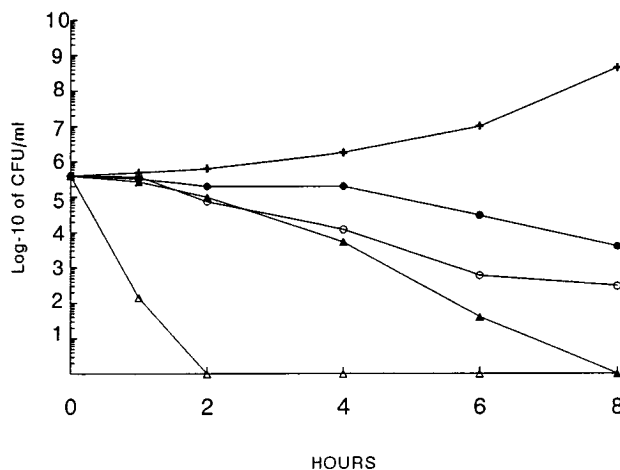

FIG. 1. Time-kill curves for *S. pneumoniae* with tobramycin (64 μg/ml = 4× the MIC) in THB (△), THB and rabbit serum (▲), THB and 25 mg of surfactant per ml (○), and THB with rabbit serum and surfactant (●). +, a growth control in THB.
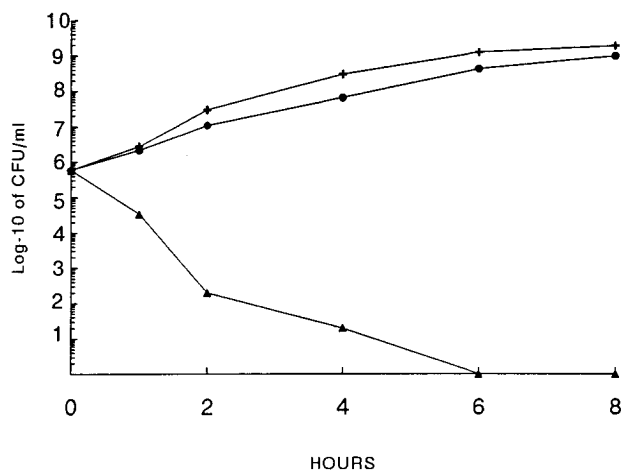
FIG. 2. Time-kill curves for *K. pneumoniae* with tobramycin (0.5 μg/ml = 1× the MIC) in MHB (▲) and MHB with 25 mg of surfactant per ml (●). +, a growth control in MHB.

falls below 7.0 (37, 47). Aminoglycoside activity is also influenced by the amount of free unbound cations in the culture medium (20, 48). Other studies have reported the inactivation of aminoglycosides in sputum, which could be explained, in part, by binding of the antibiotic to subcellular components of sputum (6, 26, 30). Aminoglycosides have been reported to interact with ribosomes, DNA, and glycoproteins in bronchial secretions (10, 34, 38).

In the present study, the partial inactivation of tobramycin cannot be explained by alterations in pH or changes in free cation concentrations after the addition of surfactant. The pH did not change after the addition of surfactant to the medium. The free cation concentration in the surfactant was not measured. However, changes in free cation concentrations should, in particular, affect *P. aeruginosa* killing by tobramycin (2, 46), whereas in the present study *P. aeruginosa* killing by tobramycin was least influenced by the surfactant. The reduced killing by tobramycin is therefore unlikely to be due to differences in free cation concentrations between the media.

To assess a possible binding of tobramycin with surfactant, we measured the amount of free drug by centrifugation-filtration. The amount of tobramycin recovered in the filtration fluid was decreased most in the presence of 25 mg of surfactant per ml. Binding of aminoglycosides to negatively charged phospholipids has previously been described as a mechanism for the nephrotoxic actions of these antibiotics (29, 31). Pulmonary surfactant comprises 90% phospholipids, mostly phosphatidylcholine and phosphatidylglycerol (44). The decreased activity of tobramycin is therefore possibly induced by the binding of tobramycin to phospholipids in the surfactant.

We have no explanation for the observed decrease in ceftazidime activity against *S. pneumoniae* in THB supplemented with rabbit serum. This decrease was found only at 4× the MIC in medium with rabbit serum. In contrast to these findings, with *S. pneumoniae* in THB there was a tendency for increased killing in the presence of surfactant; this was, however, not statistically significant.

A few studies have investigated the direct influence of pulmonary surfactant on bacterial growth (8, 18, 19, 23). Coonrod and Yoneda (8) showed that a surfactant preparation from bronchoalveolar lavage fluid of rats and, more specifically, the free fatty acids present in the surfactant caused lysis of *S. pneumoniae*. The bactericidal activity of this surfactant prepa-

ration was also observed with several other gram-positive bacteria, including viridans streptococci, *Streptococcus pyogenes*, and *Streptococcus bovis* (8). Further studies from that group showed that there was a species variation in the amount of free fatty acids recovered in the surfactant preparation of bronchoalveolar lavage fluid, which could explain the difference in *S. pneumoniae* killing between this rat surfactant and surfactant prepared from bronchoalveolar lavage fluid from guinea pigs (7). Enhancement of growth has been found for *Escherichia coli* and viridans streptococci with a crude surfactant preparation from dogs (18) and for *S. aureus* with a surfactant from rabbits (23). Studies with human alveolar lining material showed that incubation of *S. pneumoniae* or *Haemophilus influenzae* with the alveolar lining material had no effect on the viabilities of these bacteria; in fact, alveolar lining material supported the replication of *H. influenzae* (19). In the present study, no effect of surfactant on the growth of *K. pneumoniae*, *P. aeruginosa*, or *S. aureus* was found. Our results show no *S. pneumoniae* killing by surfactant. *S. pneumoniae* growth was not affected by surfactant except for a decrease in the growth rate in THB with both surfactant and rabbit serum compared with the growth rates in THB with rabbit serum. The surfactant used in our study is a highly purified extraction from bovine lungs, which could explain the observed conflicting results on *S. pneumoniae* growth. It is comparable to the commercially available surfactants which are used for the treatment of infant respiratory distress syndrome. The concentration of 25 mg/ml is based on the current surfactant concentrations used for surfactant replacement therapy (12).

Preliminary results (45a) on *S. pneumoniae* growth in the presence of surfactant showed a stimulation of growth compared with *S. pneumoniae* growth in unsupplemented THB. In the present study this could not be supported by the data. For optimal *S. pneumoniae* growth, THB is generally supplemented with serum. These preliminary results provided the rationale for studying *S. pneumoniae* killing in both THB and THB supplemented with rabbit serum. Moreover, serum is known to inhibit the functional integrity of pulmonary surfactant (15), and this, too, could interfere in a surfactant-antibiotic interaction.

In two animal models of respiratory failure caused by *Pneumocystis carinii* or influenza A virus, endotracheal instillation of exogenous surfactant restored lung function and gas exchange within 30 min (11, 45). These results, together with the histological findings in those studies, show that surfactant can reopen atelectatic areas. With a surfactant-antibiotic mixture it is expected that a large antibiotic dosage can be delivered to the lung in nonaereated areas, which are often infected areas. The use of surfactant mixed with antibiotics therefore seems to be a promising therapeutic approach in patients with severe pneumonia.

Whether the partial inactivation of tobramycin by surfactant can be compensated for by higher antibiotic dosages without an increased risk for toxic systemic levels needs further investigation in vivo. Future investigations should also focus on whether the function of surfactant remains unchanged after it is mixed with antibiotics, since this, too, could limit the use of a surfactant-antibiotic mixture.

REFERENCES

1. **Baldwin, D. R., D. Honeybourne, and R. Wise.** 1992. Pulmonary disposition of antimicrobial agents: in vivo observations and clinical relevance. Antimicrob. Agents Chemother. **36:**1176–1180.

2. **Barry, L., L. B. Reller, G. H. Miller, J. A. Washington, F. D. Schoenknect, L. R. Peterson, R. S. Hare, and C. Knapp.** 1992. Revision of standards for adjusting the cation content of Mueller-Hinton broth for testing susceptibility of *Pseudomonas aeruginosa* to aminoglycosides. J. Clin. Microbiol. **30:**585–589.

3. **Brain, J. D., D. E. Knudson, S. P. Sorokin, and M. A. Davis.** 1976. Pulmonary distribution of particles given by intratracheal instillation or by aerosol inhalation. Environ. Res. **11:**13–33.

4. **Bressolle, F., J.-E. de la Coussaye, R. Ayoub, D. Fabre, R. Gomeni, G. Saissi, J.-J. Eledjam, and M. Galtier.** 1992. Endotracheal and aerosol administration of ceftazidime in patients with nosocomial pneumonia: pharmacokinetics and absolute bioavailability. Antimicrob. Agents Chemother. **36:**1404–1411.

5. **Brown, R. B., J. A. Kruse, G. W. Counts, J. A. Russel, N. V. Christou, and M. L. Sands.** 1990. Double-blind study of endotracheal tobramycin in the treatment of gram-negative bacterial pneumonia. Antimicrob. Agents Chemother. **34:**269–272.

6. **Bryant, R. E., and D. Hammond.** 1974. Interaction of purulent material with antibiotics used to treat *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **6:**702–707.

7. **Coonrod, J. D., R. Varble, and M. C. Jarrells.** 1990. Species variation in the mechanism of killing of inhaled pneumococci. J. Lab. Clin. Med. **116:**354–362.

8. **Coonrod, J. D., and K. Yoneda.** 1983. Detection and partial characterization of antibacterial factor(s) in alveolar lining material of rats. J. Clin. Invest. **71:**129–141.

9. **Craven, D. E., L. M. Kunches, V. Kilinsky, D. A. Lichtenberg, B. J. Make, and W. R. McCabe.** 1986. Risk factors for pneumonia and fatality in patients receiving continuous mechanical ventilation. Am. Rev. Respir. Dis. **133:**792–796.

10. **Dahlberg, A. E., F. Horodyski, and P. Keller.** 1978. Interaction of neomycin with ribosomes and ribosomal ribonucleic acid. Antimicrob. Agents Chemother. **13:**331–339.

11. **Eijking, E. P., G. J. van Daal, R. Tenbrinck, A. Luijendijk, J. F. Sluiters, E. Hannappel, and B. Lachmann.** 1991. Effect of surfactant replacement on *Pneumocystis carinii* pneumonia in rats. Intensive Care Med. **17:**475–478.

12. **Fujiwara, T., and B. Robertson.** 1992. Pharmacology of exogenous surfactant, p. 561–592. *In* D. Robertson, L. M. G. Van Golde, and J. J. Batenburg (ed.), Pulmonary surfactant. Elsevier Science Publishers B.V., Amsterdam.

13. **Gommers, D., C. Vilstrup, J. A. H. Bos, A. Larsson, O. Werner, E. Hannappel, and B. Lachmann.** 1993. Exogenous surfactant therapy increases static lung compliance and cannot be assessed by measurements of dynamic compliance alone. Crit. Care Med. **21:**567–574.

14. **Hodson, M. E., A. R. L. Penketh, and J. C. Batten.** 1981. Aerosol carbenicillin and gentamicin treatment of *Pseudomonas aeruginosa* infection in patients with cystic fibrosis. Lancet **ii:**1137–1139.

15. **Holm, B. A.** 1992. Surfactant inactivation in adult respiratory distress syndrome, p. 665–684. *In* D. Robertson, L. M. G. Van Golde, and J. J. Batenburg (ed.), Pulmonary surfactant. Elsevier Science Publishers B.V., Amsterdam.

16. **Ilowite, J. S., J. D. Gorvoy, and G. C. Smaldone.** 1987. Quantitative deposition of aerosolized gentamicin in cystic fibrosis. Am. Rev. Respir. Dis. **136:**1445–1449.

17. **Jackson, G. G.** 1983. Aminoglycoside antibiotics: resistance and toxicity—a summary. Rev. Infect. Dis. **5:**S314–S316.

18. **Jalowayski, A. A., and S. T. Giammona.** 1972. The interaction of bacteria with pulmonary surfactant. Am. Rev. Respir. Dis. **105:**236–241.

19. **Jonsson, S., D. M. Musher, A. Goree, and E. C. Lawrence.** 1986. Human alveolar lining material and antibacterial defenses. Am. Rev. Respir. Dis. **133:**136–140.

20. **Kenny, M. A., H. M. Pollock, B. H. Minshew, E. Casillas, and F. D. Schoenknect.** 1990. Cation components of Mueller-Hinton agar affecting testing of *Pseudomonas aeruginosa* susceptibility to gentamicin. Antimicrob. Agents Chemother. **17:**55–62.

21. **Kharasch, V. S., T. D. Sweeney, J. Fredberg, J. Lehr, A. I. Damokosh, M. E. Avery, and J. D. Brain.** 1991. Pulmonary surfactant as a vehicle for intratracheal delivery of technetium sulfur colloid and pentamidine in hamster lungs. Am. Rev. Respir. Dis. **144:**909–913.

22. **Klastersky, J., F. Carpentier-Meunier, L. Kahan-Coppens, and J. P. Thijs.** 1979. Endotracheally administered antibiotics for gram-negative bronchopneumonia. Chest **75:**586–591.

23. **LaForce, F. M., and D. S. Boose.** 1981. Sublethal damage of *Escherichia coli* by lung lavage. Am. Rev. Respir. Dis. **124:**733–737.

24. **Laube, B. L., J. M. Links, N. D. LaFrance, H. N. Wagner, and B. J. Rosenstein.** 1989. Homogeneity of bronchopulmonary distribution of ⁹⁹ᵐTc aero-

25. **Le Conte, P., G. Potel, P. Peltier, D. Horeau, J. Caillon, M.-E. Juvin, M.-F. Kergueris, D. Bugnon, and D. Baron.** 1993. Lung distribution and pharmacokinetics of aerosolized tobramycin. Am. Rev. Respir. Dis. **147:**1279–1282.

26. **Levy, J., A. L. Smith, M. A. Kenny, B. Ramsey, and F. D. Schoenknecht.** 1983. Bioactivity of gentamicin in purulent sputum from patients with cystic fibrosis or bronchiectasis: comparison with activity in serum. J. Infect. Dis. **148:**1069–1076.

27. **Liu, M., L. Wang, E. Li, and G. Enhorning.** 1991. Pulmonary surfactant will secure free airflow through a narrow tube. J. Appl. Physiol. **71:**742–748.

28. **Lorian, V. (ed.).** 1986. Antibiotics in laboratory medicine. The William & Wilkins, Co., Baltimore.

29. **Lullmann, H., and B. Vollmer.** 1982. An interaction of aminoglycoside antibiotics with Ca binding to lipid monolayers and to biomembranes. Biochem. Pharmacol. **31:**3769–3773.

30. **Mendelman, P. M., A. L. Smith, J. Levy, A. Weber, B. Ramsey, and R. L. Davis.** 1985. Aminoglycoside penetration, inactivation, and efficacy in cystic fibrosis sputum. Am. Rev. Respir. Dis. **132:**761–765.

31. **Mingeot-Leclercq, M. P., G. Laurent, and P. M. Tulkens.** 1988. Biochemical mechanism of aminoglycoside induced inhibition of phosphatidylcholine hydrolysis by lysosomal phospholipases. Biochem. Pharmacol. **37:**591–599.

32. **Montgomery, A. B., R. J. Debs, J. M. Luce, K. J. Corkery, J. Turner, E. N. Brunette, E. T. Lin, and P. C. Hopewell.** 1987. Aerosolized pentamidine as sole therapy for *Pneumocystis carinii* pneumonia in patients with acquired immunodeficiency syndrome. Lancet **ii:**480–483.

33. **National Committee for Clinical Laboratory Standards.** 1990. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A2, 2nd ed. National Committee for Clinical Laboratory Standards, Villanova, Pa.

34. **Potter, J. L., L. W. Matthews, S. Spector, and J. Lemm.** 1965. Complex formation between basic antibiotics and deoxyribonucleic acid in human pulmonary secretions. Pediatrics **36:**714–720.

35. **Ramsey, B. W., H. L. Dorkin, J. D. Eisenberg, R. L. Gibson, I. R. Harwood, R. M. Kravitz, D. V. Schidlow, R. W. Wilmott, S. J. Astley, M. A. McBurnie, K. Wentz, and A. L. Smith.** 1993. Efficacy of aerosolized tobramycin in patients with cystic fibrosis. N. Engl. J. Med. **328:**1740–1746.

36. **Reimer, L. G., C. W. Stratton, and L. B. Reller.** 1986. Minimum inhibitory and bactericidal concentrations of 44 antimicrobial agents against three standard control strains in broth with and without human serum. Antimicrob. Agents Chemother. **19:**1050–1055.

37. **Rubenis, M., V. M. Kozij, and G. G. Jackson.** 1963. Laboratory studies on gentamicin. Antimicrob. Agents Chemother. **3:**153–156.

38. **Saggers, B. A., and D. Lawson.** 1968. In vivo penetration of antibiotics into sputum in cystic fibrosis. Arch. Dis. Child. **43:**404–409.

39. **SAS Institute Inc.** 1990. SAS users guide. SAS Institute Inc., Cary N.C.

40. **Spencer, H.** 1985. The bacterial pneumonias, p. 176–213. *In* Pathology of the lung. Pergamon Press, Inc., Elmsford, N.Y.

41. **Tisdale, J. E., M. T. Pasko, and J. M. Mylotte.** 1989. Antipseudomonal activity of simulated infusions of gentamicin alone or with piperacillin assessed by serum bactericidal rate and area under the killing curve. Antimicrob. Agents Chemother. **33:**1500–1505.

42. **Tulkens, P. M.** 1991. Pharmacokinetic and toxicological evaluation of a once-daily regimen versus conventional schedules of netilmicin and amikacin. J. Antimicrob. Chemother. **27:**S49–S61.

43. **Unertl, K. E., F. P. Lenhart, H. Horst, and K. Peter.** 1992. Systemic antibiotic treatment of nosocomial penumonia. Intensive Care Med. **18:**S28–S34.

44. **Van Golde, L. M. G., J. J. Batenburg, and B. Robertson.** 1988. The pulmonary surfactant system: biochemical aspects and functional significance. Physiol. Rev. **68:**374–454.

45. **Van Daal, G. J., J. A. H. Bos, E. P. Eijking, D. Gommers, E. Hannappel, and B. Lachmann.** 1992. Surfactant replacement therapy improves pulmonary mechanics in end-stage influenza A pneumonia in mice. Am. Rev. Respir. Dis. **145:**859–863.

45a.**van't Veen, A., J. W. Mouton, D. Gommers, J. A. J. W. Kluytmans, P. Dekkers, and B. Lachmann.** Unpublished data.

46. **Washington, J. A., R. J. Snyder, P. C. Kobner, G. G. Wiltse, D. M. Ilstrup, and J. T. McCall.** 1978. Effect of cation content of agar on the activity of gentamicin, tobramycin, and amikacin against *Pseudomonas aeruginosa*. J. Infect. Dis. **137:**103–111.

47. **Young, L. S., and W. L. Hewitt.** 1973. Activity of five aminoglycoside antibiotics in vitro against gram-negative bacilli and *Staphylococcus aureus*. Antimicrob. Agents Chemother. **4:**617–625.

48. **Zimelis, V. M., and G. G. Jackson.** 1973. Activity of aminoglycoside antibiotics against *Pseudomonas aeruginosa*: specificity and site of calcium and magnesium antagonism. J. Infect. Dis. **127:**663–669.