

## Analysis of Vancomycin Entry into Pulmonary Lining Fluid by Bronchoalveolar Lavage in Critically Ill Patients

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Received 29 June 1992/Accepted 15 November 1992

Vancomycin penetration into the fluid lining the epithelial surface of the lower respiratory tract was studied by performing fiberoptic bronchoscopy with bronchoalveolar lavage on 14 critically ill, ventilated patients who had received the drug for at least 5 days. The apparent volume of epithelial lining fluid (ELF) recovered by bronchoalveolar lavage was determined by using urea as an endogenous marker. Vancomycin levels in ELF ranged from 0.4 to 8.1 µg/ml (mean, 4.5 µg/ml), while the mean simultaneous level of the drug in plasma was 24 µg/ml (range, 9 to 37.4 µg/ml). There was a significant relationship ( $r = 0.64$ ,  $P < 0.02$ ) between vancomycin levels in plasma and those in ELF, with a correlation whose slope (0.15) indicated that the blood-to-ELF ratio of drug penetration was 6:1. Using the albumin concentration in ELF as a marker of lung inflammation, we found that vancomycin penetration was higher in patients with ELF albumin values of  $\geq 3.4$  mg/ml than in patients with normal values ( $< 3.4$  mg/ml) ( $P < 0.02$ ). These results suggest that the vancomycin distribution includes the ELF of the lower respiratory tract at a concentration that is dependent upon the levels in blood and the alveolar capillary membrane protein permeability. These concentrations were well above the MICs for most staphylococci and enterococci.

Vancomycin, a glycopeptide antibiotic, is used worldwide to treat deep-seated gram-positive bacterial infections caused by staphylococci or enterococci resistant to  $\beta$ -lactams or patients with significant allergy to  $\beta$ -lactams (18, 29). These pathogens may be responsible for nosocomial pneumonia, which is a common and life-threatening problem complicating the management of patients receiving mechanical ventilation (24). Successful treatment of bacterial pneumonia will, however, depend upon adequate delivery of the antibiotic to the area of infection. Unfortunately, little is known about the penetration of vancomycin into lung tissue.

With the advent of the technique of bronchoalveolar lavage (BAL), it is now possible to directly obtain a sample of the fluid and cells lining the epithelial surface of the human lower respiratory tract (4, 21). Therefore, in an attempt to quantify the penetration of vancomycin into lung alveoli, we obtained both BAL fluid and blood samples from critically ill patients who were receiving this antibiotic as part of their therapy. In addition, we investigated whether vancomycin penetration was modified by the inflammatory status of the lung.

(The results were presented in part at the 31st Interscience Conference on Antimicrobial Agents and Chemotherapy [13a].)

### MATERIALS AND METHODS

**Patient population and therapeutic protocol.** The study population consisted of 19 critically ill patients in the acute phase of severe infection caused by gram-positive cocci, as documented by positive blood cultures or perioperative specimen cultures (acute mediastinitis after cardiac surgery,  $n = 8$ ; prosthetic or native valve endocarditis,  $n = 3$ ; catheter-related septicemia,  $n = 8$ ). All of them were me-

chanically ventilated and, because of the presence of a new pulmonary infiltrate with fever and/or purulent tracheal secretions, underwent flexible fiberoptic bronchoscopy with a protected specimen brush (PSB) and BAL a few days later, after the initiation of treatment with vancomycin. For each patient, the following clinical variables were recorded: age, sex, weight, disease severity score on admission (as assessed by the APACHE II score) (13), creatinine clearance, and lung injury score as previously described by Murray et al. (17). Each patient had been receiving vancomycin for at least 5 days. The antibiotic was administered intravenously over 2 h at an initial dose of 15 mg/kg of body weight and was adjusted thereafter in order to obtain trough concentrations of about 15 to 20 µg/ml. Informed consent was obtained from all patients or from the nearest relative, and the procedures used were in accordance with the ethical standards of the Committee for the Protection of Human Subjects at our hospital.

**Bronchoscopy.** Fiberoptic bronchoscopy with BAL was performed after premedication with intravenous phenoperidine and a short-acting paralytic agent, just before the next planned injection of vancomycin, at a time approximating the minimal concentration of vancomycin in plasma. A flexible fiberoptic bronchoscope was introduced through an indwelling endotracheal tube by using a special adaptor. After routine inspection of the respiratory tract and aspiration of tracheobronchial secretions, the bronchoscope tip was advanced to the bronchial orifice of a lung segment identified radiologically as that containing the new infiltrate. A PSB (model BWF/10/70/90; Meditech, Watertown, Mass.) was then advanced to a subsegmental, peripheral position, after dislodging the distal catheter plug, to obtain lower airway secretions for microbial cultures. After the brushing, the bronchoscope was positioned in the adjacent subsegment, and lavage was performed by infusing five 20-ml aliquots of sterile 0.9% saline through the aspiration port and

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retrieving them immediately via the same port by gentle suction. The liquid recovered after the first aliquot was considered representative of a bronchial wash and was discarded. The remaining lavage fluid was pooled. The time that elapsed between the beginning of BAL and the total recovery of the five aliquots was kept as short as possible, to minimize free diffusion of solutes, particularly urea, through the alveolar epithelium during the procedure. Blood samples were drawn at the end of the bronchoscopy, and plasma was promptly separated and frozen at  $-70^{\circ}\text{C}$  until assayed. All bronchoscopy specimens were transported to the laboratory within 5 min of collection.

**Bacteriologic and cytologic studies.** Prior to any centrifugation, total cell counts were made on a portion of the original lavage fluid. Cyto centrifuge slides were prepared with aliquots of the original lavage fluid containing  $5 \times 10^4$  cells by using a Cytospin 2 cyto centrifuge (Shandon Southern Products, Cheshire, England) and were stained with a Wright-Giemsa-type stain. Neutrophils, macrophages, and lymphocytes were recorded as the percentage of total leukocytes. Bronchial epithelial cells were counted and recorded as the percentage of total cells. In addition, 300 cells were examined at a magnification of  $\times 1,000$  and the percentage of cells containing intracellular microorganisms and the average number of extracellular bacteria per oil-immersion field were determined. Lavage fluid samples were then centrifuged at  $4^{\circ}\text{C}$  (5 min,  $800 \times g$ ) to separate cells from the fluid components. Cell pellets and fluid supernatant were stored at  $-70^{\circ}\text{C}$  until analyzed.

PSB samples were subjected to bacteriologic examination as described in detail elsewhere (8). A diagnosis of pneumonia was retained either when quantitative cultures of PSB samples yielded  $\geq 10^3$  CFU/ml (8) or when microscopic examination of BAL showed that more than 5% of the cells contained intracellular microorganisms (7).

**Exclusion criteria.** Patients in whom one or more of the following criteria were fulfilled were excluded from the study: (i) patients with severe pulmonary abnormalities as defined by a lung injury score equal to or greater than 2.5; (ii) BAL procedure duration of  $>2$  min; (iii) proportion of bronchial epithelial cells in BAL fluid of  $>2\%$  of total cells; (iv) erythrocyte contamination with more than five cells per oil-immersion field.

**Determination of  $V_{\text{ELF}}$  recovered by BAL.** To quantify the apparent epithelial lining fluid (ELF) volume ( $V_{\text{ELF}}$ ) obtained by BAL, we used urea as an endogenous marker of ELF dilution, as described by Rennard et al. (19). Since urea diffuses readily throughout the body, the urea concentrations in ELF and plasma are the same (26, 27). In this setting, if the concentration of urea in plasma and the quantity of urea in a lavage sample are known, the volume of ELF obtained can be calculated as follows:  $V_{\text{ELF}} = (\text{volume of BAL} \times [\text{urea}] \text{ in BAL}) / [\text{urea}] \text{ in plasma}$ , where [urea] is the concentration of urea. Once the recovered  $V_{\text{ELF}}$  is known, then any acellular component (e.g., albumin or vancomycin) can be referenced to the  $V_{\text{ELF}}$  from which it was obtained. The urea content of BAL fluid samples was determined by using a commercially available kit, as described previously (19). The albumin concentrations in BAL fluid and plasma were determined by enzyme-linked immunoassay (20).

**Vancomycin assay.** Vancomycin concentrations in the BAL fluid supernatant were determined by reverse-phase liquid chromatography coupled to a spectrophotometer. Separation was performed on a 5- $\mu\text{m}$ -particle-size Ultrasphere ODS column (250 by 4.6 mm (Beckman, Gagny,

France) protected by a precolumn (Brownlee Spheri 5 RP 18; 30 by 4.6 mm; Applied Biosystems). The mobile phase was a mixture of acetonitrile-tetrahydrofuran-0.2% triethylamine phosphate (pH 3.2; 5.5/0.8/93.7; vol/vol/vol) with a flow rate of 2 ml/min. Two procedures were used to optimize sensitivity: (i) the wavelength was set at 229 nm, which gave a molecular extinction coefficient 8-fold greater than that obtained at 254 nm, and (ii) BAL fluid supernatant was lyophilized and the powder was suspended in 1 ml of  $10^{-3}$  M hydrochloric acid to obtain a solution that was 25-fold more concentrated than the original solution. Linear regression analysis of the standard calibration lines of  $10^{-3}$  M HCl solutions gave a correlation coefficient of 0.998, indicating the excellent linearity of the assay between 0 and 3  $\mu\text{g/ml}$ . Intraassay and interassay coefficients of variation were 3.6% ( $n = 5$ ) and 7.8% ( $n = 5$ ), respectively, for  $10^{-3}$  M HCl samples spiked to 1  $\mu\text{g/ml}$  with vancomycin. The assay's sensitivity measured in the  $10^{-3}$  M HCl solution was 0.2  $\mu\text{g/ml}$ , corresponding to 0.008  $\mu\text{g}$  of vancomycin per ml of BAL fluid. The limit of detection was 10 ng of vancomycin when we used a 75- $\mu\text{l}$  sample. A fluorescence polarization immunoassay, described previously (23), was used to measure vancomycin concentrations in plasma.

**Statistical analysis.** All data are expressed as means  $\pm$  standard deviations. Statistical methods included linear correlation and the Mann-Whitney nonparametric U test. All results were subjected to statistical analysis, including those noted as being below the lower limit of sensitivity, in which case the lowest value detectable in that assay was used. A *P* value of less than 0.05 was considered significant.

## RESULTS

BAL was performed safely in all 19 patients who were enrolled in the protocol. Of these 19 patients, 5 were excluded from the study according to the criteria specified in the protocol. In one case, the time needed to collect BAL fluid was greater than 2 min, in two cases, the BAL fluid was highly contaminated with bronchial wash (proportion of bronchial epithelial cells, 18 and 12%, respectively), and in two cases, the BAL fluid was markedly bloody. The demographic characteristics of the study participants are given in Table 1. The subjects, 11 males and 3 females, ranged in age from 22 to 79 years (mean,  $60 \pm 16$  years), and their weights ranged from 42 to 113 kg (mean,  $74 \pm 17$  kg). They had a mean APACHE II score of  $18.7 \pm 6$  (range, 9 to 32) and a mean creatinine clearance of  $50.6 \pm 32.9$  ml  $\text{min}^{-1}$  (range, 13 to 123 ml  $\text{min}^{-1}$ ). The mean duration of treatment with vancomycin before the protocol was  $6.6 \pm 1.75$  days (range, 5 to 11 days), and patients had received a cumulative dose of  $9.4 \pm 4.5$  g of vancomycin (range, 3 to 17.5 g). The bronchoscopy procedure was performed  $18.4 \pm 11$  h (range 8 to 50 h) after the last injection of vancomycin and just before the next planned injection. BAL was completed within a mean time of 1 min 15 s (range, 1 min 5 s to 1 min 50 s), and therefore, the average dwell time of fluid in the lung must have been  $<1$  min. On the basis of the criteria defined above, only two patients (patients 2 and 6 in Table 1) had pneumonia at the time of evaluation; the other patients were undergoing mechanical ventilation for acute respiratory failure caused by postoperative pulmonary edema or atelectasis ( $n = 8$ ), chronic obstructive lung disease ( $n = 1$ ), or neurologic problems ( $n = 3$ ).

**Characterization of the cell population and  $V_{\text{ELF}}$  recovered by BAL.** The volume recovered by BAL was  $31 \pm 8$  ml, with a mean total number of cells of  $262 \times 10^3/\text{ml}$  of lavage fluid.

TABLE 1. Demographic and clinical characteristics of patients<sup>a</sup>

Patient no.	Age (yr)	Sex <sup>b</sup>	Wt (kg)	CL <sub>CR</sub> (ml min <sup>-1</sup> ) <sup>c</sup>	APACHE II score	PaO <sub>2</sub> /FIO <sub>2</sub> (mm Hg) <sup>d</sup>	Lung injury score <sup>e</sup>	Albumin in ELF (mg/ml)
1	54	M	88.3	78	14	185	1.33	3.4
2	56	F	82	29	17	273	0.66	1.2
3	68	M	113	44	27	264	1.33	0.8
4	78	F	82	26	14	193	1	1.2
5	79	M	75	24	20	134	1.66	2.9
6	22	M	58.3	91	23	237	1	3.0
7	58	M	68.2	82	15	242	0.66	5.4
8	63	M	62.2	13	18	184	1.33	5.0
9	71	M	65	54	15	203	1.75	5.4
10	34	F	42.1	36	32	327	1.75	1.3
11	70	M	87.3	17	20	207	1.66	3.6
12	66	M	84.1	123	15	189	1	2.3
13	67	M	67	66	23	114	1.66	3.6
14	53	M	63.2	26	9	79	1.66	5.5
Mean ± SD	60 ± 16		74.1 ± 17.1	50.6 ± 32.9	18.7 ± 6	202 ± 65	1.3 ± 0.4	3.2 ± 1.7

<sup>a</sup> Patients 2 and 6 had pneumonia at the time of BAL evaluation.

<sup>b</sup> M, male; F, female.

<sup>c</sup> CL<sub>CR</sub>, creatinine clearance.

<sup>d</sup> PaO<sub>2</sub>, arterial oxygen pressure; FIO<sub>2</sub>, inspired oxygen fraction.

<sup>e</sup> This score (17) is based on a four-component system including a chest roentgenogram score, a hypoxemia score, a positive end-expiratory pressure score, and a respiratory system compliance score, with each score graded from 0 to 4. The final value is obtained by dividing the aggregate sum by 4. Possible scores range from 0 to 4, with the higher scores indicating greater severity of lung injury.

The differential cell count revealed 42% ± 32% macrophages, 54% ± 35% neutrophils, and 4% ± 6% lymphocytes. The proportion of bronchial epithelial cells was <2% in all patients. Erythrocyte contamination of the cell pellets was not observed. On the basis of the measurements of the total amount of urea in the lavage fluid recovered by an 80-ml lavage and the concentration of urea in plasma, the calculated V<sub>ELF</sub> recovered averaged 0.56 ± 0.54 ml (range, 0.18 to 1.81 ml). When urea was used as a marker of ELF dilution in BAL, the albumin concentration in ELF was calculated to be 3.23 ± 1.7 mg/ml (range, 0.8 to 5.5 mg/ml). This value represented 12% ± 5.7% of the albumin concentration in the plasma of the same individuals.

**Pulmonary distribution of vancomycin.** The vancomycin concentrations in the plasma and ELF of the lower respiratory tracts of the study patients are given in Table 2. The mean vancomycin level in ELF (4.5 ± 2.3 µg/ml) represented 18% of the simultaneous levels in plasma (24 ± 10 µg/ml). In one patient (patient 5 in Table 2), although the

drug was detected in BAL fluid, it could not be quantified accurately because the level was below the limit of sensitivity of the assay; therefore, for this patient, the lowest value detectable by this assay was used for the statistical analysis. A significant relationship existed (r = 0.64, P < 0.02) between vancomycin levels in plasma and those in ELF (Fig. 1).

Using the albumin concentration in ELF as a marker of lung inflammation, we divided the study population into two groups of patients. The first one consisted of the seven patients with normal albumin concentrations in ELF (<3.4 mg/ml) (19), and the second one had seven patients with high albumin concentrations (≥3.4 mg/ml) in ELF. As shown in Fig. 1, in patients with more pronounced lung inflammation, drug penetration into the lung tended to be better than that in the other patients. Because of the individual variation of the values observed, the Mann-Whitney nonparametric U test was applied as the appropriate method for testing this

TABLE 2. Distribution of vancomycin in 14 critically ill patients

Patient no.	Vancomycin concn (µg/ml) in:	
	Plasma	ELF
1	10.9	2.5
2	33.5	5.6
3	37.4	4.2
4	25.2	4.5
5	17.3	0.4
6	31.4	4.2
7	13.9	3.9
8	31.7	6.3
9	13.9	5.6
10	28.4	8.1
11	33.9	7.5
12	15.9	1.4
13	9	1.8
14	32.9	6.3
Mean ± SD	24 ± 10	4.5 ± 2.3

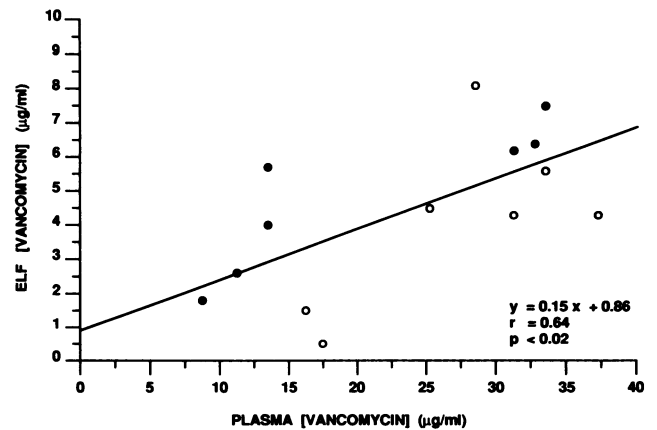


FIG. 1. Relationship between vancomycin concentrations in plasma and ELF. Symbols: ○, patients with albumin level in ELF of <3.4 mg/ml; ●, patients with albumin levels in ELF of ≥3.4 mg/ml.

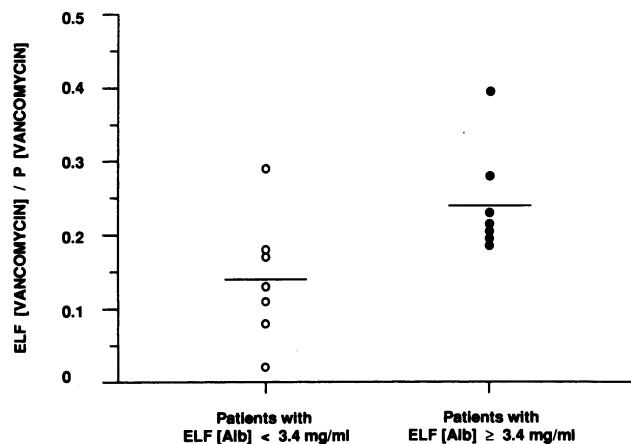


FIG. 2. Vancomycin penetration into ELF as assessed by the ratio of the vancomycin concentration in ELF to that in plasma ([vancomycin] in ELF/[vancomycin] in plasma) in patients with a low albumin concentration in ELF (<3.4 mg/ml) (○) and patients with a high albumin concentration in ELF ( $\geq$ 3.4 mg/ml) (●).

hypothesis. Using this test, we found that vancomycin penetration into ELF, as assessed by the ratio of vancomycin concentration in ELF to that in plasma, was significantly increased in patients with lung inflammation (mean [vancomycin] in ELF/[vancomycin] in plasma ratio in patients with high ELF albumin concentrations, 24.6% [range, 42.6 to 19.2%], versus 14% [range, 2.3 to 28.5%] in patients with normal ELF albumin concentrations [ $P < 0.02$ ]) (Fig. 2).

## DISCUSSION

Despite over 20 years of clinical administration of vancomycin, little is known regarding the distribution of the drug in lung tissue in humans. Results of studies in animals must be interpreted with caution, since there are many interspecies differences in vancomycin pharmacology, especially protein binding (1, 2, 15). In humans, most previous studies of antibiotic penetration into the respiratory tract have been based on comparisons of antibiotic levels in blood and bronchial secretions (5). However, as far as parenchymal infections are concerned, the clinical significance of the antibiotic concentration in bronchial secretions is questionable. Therefore, it is not known how well vancomycin enters the lung acini, the site of the pneumonic process. In the present study, the pulmonary distribution of vancomycin was directly assessed by BAL, a procedure whereby the cells and fluid in contact with the alveolar epithelial lining can be harvested at a high degree of purity.

BAL has become an interesting technique for detecting a variety of pulmonary disorders and studying the concentrations of various solutes in the epithelial fluid that lines the surface of the lung (3, 4, 6, 11, 20, 21, 28). The amount of BAL fluid injected during the procedure has not been standardized. The BAL Cooperative Group Steering Committee (3) has recommended using 240 ml for the evaluation of patients with interstitial lung disease and healthy individuals. In studies that use BAL for assessment of critically ill patients, amounts of BAL fluid have ranged from 100 to 300 ml (21). At least 100 to 120 ml is probably necessary for retrieving secretions from the periphery of the lung subsegment that is lavaged, as assessed by digital subtraction radiography and studies with sequential lavage (12, 21).

Because it is probable that the smaller aliquots do provide adequate samples of the alveolar space, especially after the first one is given, and in order to minimize the risk of the procedure, we chose to use five aliquots of 20 ml each to perform BAL in the present study.

During BAL, the fluid lining the surfaces of the lung is diluted by the solution instilled into the airways. One approach to quantitating the dilution of ELF by BAL is to measure in the BAL the concentration of a solute that is normally present at the same concentration in the ELF and plasma. Rennard and coworkers (19) introduced the use of urea for this purpose. Because of the low molecular weight of urea and the relative rapidity with which this solute diffuses across cell membranes, it can be postulated that concentrations in ELF and plasma are similar (26, 27). With this approach, the total volume of ELF recovered can be calculated by simple dilution principles. Once the recovered  $V_{\text{ELF}}$  is known, it is possible to estimate the actual concentration of molecules in ELF in situ, and thus to determine whether the concentrations of the various molecules are relevant to pharmacological activity or biological function. In addition, it is likely that diseases that alter the alveolar capillary membrane would not change the permeability of urea. Thus, the concentration of urea in ELF would continue to equal that in plasma, allowing estimation of ELF recovery and ELF protein concentrations in patients with lung disease, as well as in normal subjects.

An ideal indicator for estimating the dilution of ELF by BAL has, however, yet to be described. Preliminary studies in animals suggest that small relatively diffusible indicators such as urea tend to leak into the air spaces during the lavage procedure and therefore falsely elevate the indicator concentration in the BAL fluid (9, 10, 14). The result is an overestimate of ELF volume and an underestimate of the concentrations of proteins and other molecules in the in situ ELF (4). This presumably would be a greater problem if the duration of lavage was increased or the permeability of the pulmonary epithelium was considerably increased by clinical or experimental injuries (10, 14, 19). To circumvent this problem in our study, we chose not to include in the protocol patients with overt clinical pulmonary abnormalities, such as patients with acute respiratory distress syndrome or patients with a lung injury score of greater than 2, and we used a rapid lavage technique, as described by Rennard and coworkers (19), in which the procedure of instillation and collection was completed in less than 2 min. Because the contact time between the instilled saline and the alveolar milieu is necessarily lower than the delay time between the beginning of instillation and the recovery of fluid, the average dwell time of fluid in the lung was probably less than 30 s in our patients (10, 28). It is therefore possible that the vancomycin concentration in the recovered ELF was affected by diffusion of urea from the interstitium and blood into the airspace fluid, but we postulate that it did not markedly modify the results. As a matter of fact, the calculated extracellular lining fluid volumes of  $0.56 \pm 0.54$  ml for a 80-ml lavage seem to be in the appropriate range anticipated for this fluid (10, 14, 19, 21).

Because patients who were receiving vancomycin as part of their therapy were included in the present study only if they were clinically suspected of having developed nosocomial pneumonia, we could not control the total duration of treatment with this drug before evaluation by BAL. Whether we were able to achieve steady-state or near-steady-state conditions in all patients remains, therefore, uncertain, especially in the subgroup of eight patients with impaired

renal function (creatinine clearance, <50 ml/min), since the vancomycin half-life is quite variable in this setting and may be as long as 17 days (29). Three findings, however, suggest that it is very unlikely that a period of treatment with vancomycin that was too short would have influenced our results. First, all patients had been receiving vancomycin for at least 5 days (mean duration of treatment before BAL,  $6.6 \pm 1.75$  days; range, 5 to 11 days). Second, no correlations were found between vancomycin levels in ELF and the duration of treatment or the cumulative doses of vancomycin received by the patients (data not shown). Finally, retrospective analysis of vancomycin doses, time intervals between injections, and actual vancomycin trough levels in plasma by using a software package designed to predict vancomycin levels in plasma according to pharmacokinetic parameters of a population in an intensive care unit and their own creatinine clearance (Abbottbase Pharmacokinetic System; Abbott Laboratories) demonstrated that the duration of treatment was greater than five elimination half-lives in all but two patients, in whom it was 3 and 4.5 times greater.

In the present study, we found that increasing vancomycin levels in the blood led to an increase in the pulmonary ELF levels, with a correlation whose slope (0.15) suggested a 6:1 ratio between ELF levels in blood and lung. Accordingly, if the level of vancomycin in blood and the MIC of vancomycin for the infecting organism are known, a reasonable estimate of the ratio of the in situ antibiotic concentration to the MIC can be made. The MICs of vancomycin against *Staphylococcus aureus*, *Staphylococcus epidermidis*, streptococci, and enterococci are usually in the range of 0.25 to 2  $\mu\text{g/ml}$  (29). The ELF vancomycin concentrations were above these values in all patients but one. Furthermore, all patients with plasma vancomycin levels of  $\geq 20 \mu\text{g/ml}$  had ELF levels that were 2 to 30 times the MICs for most of the gram-positive organisms, suggesting that one of the therapeutic goals in patients with severe pneumonia caused by one of these microorganisms would be to obtain trough levels of vancomycin in plasma of at least 20  $\mu\text{g/ml}$ .

Vancomycin in human plasma is moderately bound to proteins and especially to albumin, with a free fraction of ca. 55% (1, 2). If there were no proteins in the ELF, one would expect the concentration of the free drug to be at this level, i.e., a concentration as high as 55% of the concentration in plasma, because the free fraction alone is able to penetrate into the ELF. As demonstrated by the present and previous studies (6, 10, 19, 28), small amounts of plasma proteins normally reach the alveolar epithelial surface by a size-selective process that restricts the passage of very large molecules. In the present study, we found that the albumin concentration in the ELF was 12% of its level in plasma, a value very similar to that reported by others (6, 19). One would therefore expect an even higher (i.e., >55% of the level in plasma) concentration of vancomycin in the ELF. Several mechanisms may, however, restrict the passage of vancomycin through the alveolar capillary barrier. First, the alveolar epithelial cells that are tightly apposed by numerous zonulae occludens may not be completely penetrable by nonlipophilic drugs, such as vancomycin, and therefore may not allow the passage of such antibiotic molecules (4, 10, 26, 27). Second, certain types of cells that constitute the blood-bronchoalveolar barrier have a specific metabolic function and can be responsible for metabolization and local degradation of the drug (5, 10). Finally, the degree of vancomycin ionization may further restrict the transport of the drug to ELF, because this antibiotic is positively charged (18).

These facts may explain why, in our study, vancomycin concentrations in ELF were only 18% of those in plasma.

Clinical studies have suggested that modifications of the albumin concentration in ELF are linked to the presence of an inflammatory process in the lower respiratory tract (11, 21, 22, 28). A higher alveolar/plasma albumin ratio during an inflammatory process may be the consequence of increased transepithelial passage and/or decreased metabolism of albumin. In the present study, the increased albumin concentration in ELF was associated with an enhancement of vancomycin penetration into the alveoli. This observation indicates an increase in both bound and unbound fractions of the drug, since they are in equilibrium. It is widely accepted that only the free fraction can act against bacteria, but the direct effects of protein binding have not been clearly established (25). However, it may be that the protein-bound fraction, although not directly active, may act as a readily available reservoir, as has been suggested for other antibiotics (16).

In summary, the data presented herein demonstrated that vancomycin is distributed to the pulmonary ELF at a concentration that is dependent upon levels in blood and alveolar capillary membrane permeability. They also showed that this antibiotic was present in the lung in concentrations largely exceeding the MICs of the drug for most gram-positive cocci when levels in blood had reached 20  $\mu\text{g/ml}$ .

#### ACKNOWLEDGMENTS

We are indebted to A. Meulemans for helpful suggestions, to P. Loiseau for skillful technical assistance, and to C. Brun and C. Largenton for preparing the manuscript.

This work was supported in part by a grant from Lilly Laboratories, Saint-Cloud, France.

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