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Vancomycin Entry into Lung Lymph in Sheep

D. GAIL MAY,[1] CHARLES W. STRATTON,[2] WILLIAM D. DENNEY,[3] FLORENCE L. WATTS,[3] GORDON R. BERNARD,[3] AND ROBERT A. BRANCH[1]*

Departments of Pharmacology,[1] Pathology,[2] and Medicine,[3] Vanderbilt University School of Medicine, Nashville, Tennessee 37232

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The distribution of antibiotics into target tissues is a crucial factor in therapeutic efficacy. To estimate the availability of systemically administered vancomycin to the interstitial fluid in the lung, we have used a sheep model with a chronic pulmonary lymph fistula to collect simultaneously series of plasma and pulmonary lymph specimens during a 6-h period after an intravenous dose of vancomycin (7 mg/kg). After a minor delay in transit from blood to lymph, vancomycin was completely distributed to pulmonary lymph with a ratio of free drug in lymph to free drug in plasma of 0.9. This suggests that vancomycin is an excellent choice for treating pulmonary infections by susceptible organisms.

Vancomycin is a glycopeptide antibiotic widely used to treat infections caused by staphylococci and enterococci. These pathogens are capable of causing pulmonary infections (3, 4, 8). Successful treatment of bacterial pneumonia depends on adequate delivery of antibiotic into the area of infection (1, 9, 12), yet little is known about the penetration of vancomycin into lung tissue (10). Lymph is considered identical in composition to the interstitial fluid that is its source (16). Collection of lymph from major lymphatic ducts therefore provides an opportunity to measure the ability of a drug to distribute to the extravascular, extracellular compartment of the lungs. Using a sheep model featuring a chronic pulmonary lymph fistula, we have applied this approach to estimate the penetration of vancomycin into pulmonary interstitial tissue.

MATERIALS AND METHODS

Sheep model. Chronic pulmonary lymph fistulas were prepared in five young female sheep (*Ovis aries*) by the method of Staub et al. (15). The right external jugular vein and the efferent duct of the right caudal mediastinal lymph node were cannulated during a single surgery (the caudal end of this node having been ligated to cut off the inflow of systemic lymph). Sheep were allowed to recover for 5 to 7 days and were maintained in animal cages that allowed free access to food and water while protecting the catheters.

Antimicrobial agent. Vancomycin was purchased from Eli Lilly & Co., Indianapolis, Ind., in the form used clinically and was prepared for use according to instructions of the manufacturer.

Administration of vancomycin and collection of samples. A vancomycin dose of 7.0 mg/kg was used because this is comparable to the dose received by humans for clinical therapy. A single 250-mg dose of vancomycin was diluted in 250 ml of 5% glucose in sterile water and administered by intravenous infusion over a 30-min period to each of five sheep with individual weights from 30 to 40 kg. Venous blood and pulmonary lymph were collected concurrently prior to administration of vancomycin, immediately after intravenous infusion, and at 30 min and 1, 2, 4, and 8 h postinfusion. Specimens were centrifuged at 50 × *g* for 10 min; the supernatant plasma and lymph were removed and stored frozen at −70°C until assayed.

Assay of vancomycin. A fluorescence polarization immunoassay (TDX; Abbott Laboratories, North Chicago, Ill.) was used to assay specimens (13). In our laboratory, the sensitivity of the assay at 95% confidence limits was 0.60 μg/ml. The coefficient of variation over the concentration range of 7 to 35 μg was less than 0.05, and recovery with spiked samples over this concentration range was 100.8 ± 0.4%. Standards for the assay were prepared in both ovine plasma and lymph specimens.

Protein-binding assay. Equilibrium dialysis of ovine lymph and plasma at initial vancomycin concentrations of 5, 10, 20, and 30 μg/ml was performed with potassium phosphate buffer, pH 7.4, in a 37°C water bath for 4 h. The concentrations of total protein and albumin were determined by the biuret method.

Pharmacokinetic analysis. The area under the concentration-time curve, determined by the log trapezoidal method, was used to calculate vancomycin clearance, and the volume of distribution of vancomycin was determined by standard noncompartmental modeling. Terminal rate constant and terminal half-life were determined after least-squared, nonlinear, monoexponential curve fitting of the logarithm of the concentration in plasma versus time of plasma sampling, by using terminal time points. The percentage of unbound drug (F_f) was calculated from the relationship $F_f = C_u/C_t$, where F_f is the free fraction, C_u is the concentration of unbound drug, and C_t is the total concentration. Student's paired two-tailed *t* test was used to compare protein binding in plasma and in lymph.

RESULTS

Peak levels in serum were comparable to those achieved in humans after a single dose of 7.0 mg/kg (7, 11). Immediately after infusion, total (bound plus free) vancomycin concentration in lymph was approximately 65% of total concentration in plasma. After a 30-min lag, total vancomycin concentration in lymph was at least 80% of the corresponding total concentration in plasma. Elimination of vancomycin from both sheep plasma and lymph appeared to be a multiexponential process (Fig. 1). Terminal half-lives and their associated rate constants in plasma and lymph were similar. Protein concentrations were slightly lower than those of

* Corresponding author.

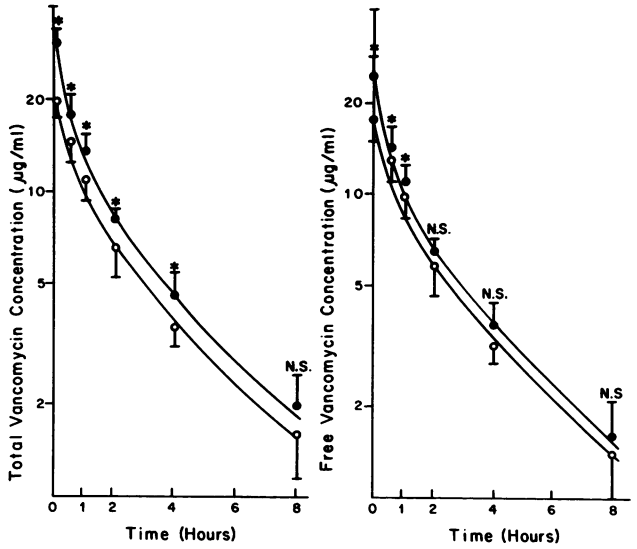

FIG. 1. Total and free-vancomycin concentrations in sheep plasma (●) and lymph (○) after intravenous infusion of vancomycin at 7.0 mg/kg (mean ± standard deviation; $n = 5$). *, $P < 0.05$. N.S., Not significant.

adult humans: total protein in plasma was 5.2 g/dl, of which 3.0 g/dl was albumin, and total protein in lymph was 2.7 g/dl, of which 2.3 g/dl was albumin. Protein binding was low in both plasma and lymph; however, the difference between the percentage of drug bound in plasma and the percentage of drug bound in lymph was significant ($P < 0.05$) (Table 1). The difference in protein binding resulted in free-vancomycin concentrations in lymph and in plasma being more similar than total concentrations were. Immediately after infusion, the ratio of free drug in lymph to free drug in plasma was 0.72, and after full distribution, the ratio was approximately 0.88 (Fig. 1).

DISCUSSION

Adequate penetration of infected tissue is a crucial factor in antibiotic efficacy. Vancomycin, the only drug in its class, is a key antibiotic in the treatment of enterococcal and staphylococcal infections, especially those due to methicillin-resistant staphylococci (14). Despite over 20 years of clinical use of vancomycin, knowledge regarding the distribution of this drug into target tissues, including lung tissues, has been lacking. The present study provides a clear illustration of rapid and complete distribution of this drug in the pulmonary interstitial fluid space.

In vivo human studies addressing the penetration of antibiotics into pulmonary interstitial tissue are difficult. Several alternative approaches, all with limitations, have been used to estimate tissue penetration. Sputum and bronchial studies show wide variations in results, possibly because of sample contamination by saliva, sampling from different parts of the lung, or variation in diffusion time. An alternative approach is a measure drug in tissue or fluid drawn from skin blisters. Interpretation of tissue cage studies and skin blister studies suffers from uncertainties in vascular supply, since areas with more blood supply are expected to have higher concentrations of the drug. Homogenized-tissue studies require grinding and disruption of tissue, which may release various solutes, cellular components, and enzymes which bind antibiotics, thereby giving misleadingly high drug levels in tissue which do not reflect free concentrations in tissue in vivo.

TABLE 1. Pharmacokinetic data for vancomycin in sheep[a]

Fluid	CL (ml/min per kg)	V (ml/kg)	$t_{1/2\beta}$ (h)	% Bound
Plasma	1.83 ± 0.36	38.4 ± 9.6	3.5 ± 1.23	18.8 ± 5.3
Lymph			3.4 ± 0.81	10.4 ± 3.7

[a] Mean ± standard deviation; obtained after intravenous infusion of vancomycin at 7.0 mg/kg. CL, Clearance; V, volume of distribution; $t_{1/2\beta}$, half-life of β phase.

In vivo animal studies offer a reasonable alternative to in vitro methods. A sheep model with a chronic pulmonary lymph fistula has been found useful in the measurement of antibiotics in lung tissue (5). The caudal mediastinal lymph node drains the bulk of pulmonary lymph in sheep; resection of the caudal end of this node eliminates systemic contamination of the lymph from the abdomen and diaphragm (15). Lymph is considered identical in composition to the interstitial fluid that is its source (16); thus, pulmonary lymph should provide an accurate assessment of concentrations of antibiotic in lung tissue (5, 6).

In the sheep model, the present study showed that vancomycin given at clinically relevant doses rapidly penetrated sheep lymph, giving adequate pulmonary interstitial concentrations over the period studied. An immediate increase of the drug concentration in plasma was associated with the appearance of antibiotic in interstitial fluid. The ratio of total vancomycin concentration in lymph to total concentration in plasma reached a maximum of 0.80 at 30 min, and this ratio was maintained for the duration of the study. Elimination of vancomycin from lymph paralleled its elimination from plasma, with similar values for terminal half-lives.

It appears from evaluation of the total (i.e., bound plus free) concentrations of vancomycin in plasma and lymph that there was significantly less vancomycin in lymph than in plasma; however, only free vancomycin is available for distribution across membranes including the vascular endothelium. When concentrations of free vancomycin in plasma and lymph were compared, this difference became statistically insignificant after 1 h. Theoretically, free-vancomycin concentrations in plasma and lymph should be equal. In this study, protein binding was determined on blanks of sheep plasma and lymph with serial concentrations of vancomycin added. If individual samples are analyzed for protein binding, it is anticipated that the ratio of free vancomycin in lymph to free vancomycin in plasma would be 1.0; however, even with the method used to determine binding, free-drug concentrations in plasma and lymph were not statistically different for the majority of observations.

The difference between the results of protein binding in plasma in this study with sheep (19%) and the known values for vancomycin protein binding in human plasma (55%) (12) may be explained by the in vitro method used or by interspecies differences. It is prudent, therefore, to be cautious in extrapolating observations with sheep to humans.

The sheep fistula model has been used previously to estimate pulmonary distribution of a single dose of four cephalosporin-like agents (5). Over an 8-h interval, ceftriaxone, cefoperazone, cefazolin, and moxalactam were well distributed. Similar to vancomycin, moxalactam exhibited a 30-min distribution lag before peak concentration in lymph was reached, after which concentrations in lymph were 70% or more of concentrations in plasma. Cefoperazone, ceftriaxone, and cefazolin distributed into lung lymph more rapidly and slightly more completely than vancomycin. Each

of these three drugs had a ratio of total concentration in lymph to total concentration in plasma of 0.75 to 1.0 after 5 min, and thereafter, ratios of concentration in lymph to concentration in plasma exceeded 1.0. Protein binding of all three drugs was low, resulting in ratios of free-drug concentration in lymph to free-drug concentration in plasma of 0.96, 0.89, 0.95, and 0.68 for cefazolin, cefoperazone, ceftriaxone, and moxalactam, respectively. These results confirm that estimation of the concentration in plasma of these drugs provides a measure of the concentration of drug in interstitial fluid at the site of a pulmonary infection.

Studies of other antibiotics by different methods imply that vancomycin and the cephalosporin series of drugs have superior pulmonary penetrations compared with those of many more commonly used agents. Sputum and bronchial-secretion studies have shown low penetrations for amoxicillin (5.7%), cephradin (15 to 20%), ampicillin (3 to 10%), cefoxitin (25%), cefuroxime (18%), and cefotaxime (25%) (1, 2, 17). The penetration of tetracycline antibiotics into bronchial secretions varies widely, as shown with minocycline (37%), doxycycline (18%), and rolitetracycline (71%). In contrast, most macrolide antibiotics show good penetration into bronchial secretions, with ratios of concentration in secretion to concentration in serum for oleandomycin, spiramycin, and clindamycin of 1.0, 1.0, and 0.62, respectively. The exception to this is erythromycin, which has a ratio of concentration in secretion to concentration in serum of only 5%. Amikacin and gentamicin bronchial-secretion penetrations have been reported to be 25 and 40%, respectively (1). It should be noted, however, that concentrations in lymph, which reflect a rapid distribution process, would not be expected to correlate with concentrations in sputum, which more closely reflect a penetration process. The issue of increased pulmonary penetration by antibiotic with inflammation remains unsettled. Wong and colleagues showed a lack of correlation between protein concentration and gentamicin, ampicillin, and cephalothin penetrations, suggesting that inflammation is unimportant (17). In contrast, by using protein concentration as a marker of inflammation, Bergogne-Berezin found a correlation between penetration and degree of inflammation for ampicillin, amoxycillin, and oleandomycin. Other antibiotics such as spiramycin, minocycline, and thiamphenicol were not affected by increased inflammation, as measured by protein concentration and grading of macroscopic purulence (1). The present study does not address the issue of vancomycin penetration in the presence of inflammation; however, because of the relatively complete penetration of the drug, additional penetration due to inflammation would probably be clinically irrelevant.

Because of variations in humoral and cellular immunity, in the extent of disease, and in organism pathogenicity, clinical response cannot be predicted by tissue penetration alone; however, treatment can obviously be optimized by choosing an antibiotic with good tissue penetration. The current study suggests that because of its rapid and relatively complete pulmonary penetration, vancomycin is a more prudent choice against susceptible pulmonary pathogens than many more commonly used antibiotics are.

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