

Pulmonary Disposition of Roxithromycin (RU 28965), a New Macrolide Antibiotic

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The penetration of roxithromycin (RU 28965), an ether oxime derivative of erythromycin, into the cells and fluid lining the epithelial surface of the lower respiratory tract was studied by performing fiber-optic bronchoscopy with bronchoalveolar lavage on eight patients who had received roxithromycin at 300 mg perorally every 12 h for 5 days. The apparent volume of epithelial lining fluid recovered by bronchoalveolar lavage was determined by using urea as an endogenous marker. There was a significant relationship ($r = 0.75$; $P < 0.02$) between roxithromycin levels in plasma and epithelial lining fluid, with a correlation whose slope suggested that the level of drug penetration into the lining fluid was 0.2. Concentrations of the antibiotic in cells recovered by bronchoalveolar lavage ($21 \pm 10 \mu\text{g/ml}$) were 2 and 10 times higher than in plasma ($11.4 \pm 5.7 \mu\text{g/ml}$) and epithelial lining fluid ($2.0 \pm 1.7 \mu\text{g/ml}$), respectively. Thus, when administered perorally in humans, roxithromycin is markedly accumulated by resident alveolar macrophages in concentrations largely exceeding the MBCs of the drug for most facultative intracellular pathogens including *Legionella pneumophila*, despite low concentrations in the epithelial lining fluid.

Roxithromycin (RU 28965), a new macrolide antibiotic, is an ether oxime derivative of erythromycin (10) with improved pharmacokinetic properties, including more predictable absorption and higher levels and prolonged residence in serum and tissue (E. Bergogne-Berezin, Abstr. 14th Int. Congr. Chemother. 1985, WS-11-6, p. 53). The drug has antimicrobial activity similar to that of erythromycin (1, 10) and is potentially active against *Legionella pneumophila*, a facultative intracellular organism (11, 19). Optimal therapy of pulmonary infections caused by such organisms would, however, require that the antibiotic penetrate in situ pulmonary phagocytes. With the advent of the technique of bronchoalveolar lavage (BAL), it is now possible to directly sample fluid and cells lining the epithelial surface of the human lower respiratory tract (9, 18). Accordingly, to quantify the penetration of roxithromycin into the lung alveoli, we obtained both BAL fluid and blood samples from patients with mild chronic obstructive pulmonary disease who were receiving this antibiotic as part of their therapy.

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MATERIALS AND METHODS

Patient population and therapeutic protocol. The study population consisted of eight patients (six male, two female; mean age, 56 ± 10 years; mean weight, 58 ± 13 kg) who were undergoing flexible fiber-optic bronchoscopy for the evaluation of mild chronic bronchitis. All of the patients had used cigarettes in the past, but two had stopped smoking at least 2 months prior to the study. The forced expiratory volume in 1 s ranged from 56 to 99% of predicted (mean 71% of predicted). Mean values for forced vital capacity and ratio of

forced expiratory volume in 1 s to forced vital capacity were $83 \pm 14\%$ of predicted and $64 \pm 8\%$, respectively.

Each patient received two 150-mg tablets of roxithromycin perorally every 12 h for a total of 10 doses. Medications were administered at 8:00 a.m. and 8:00 p.m. A final 300-mg dose was given on the morning of day 6, 2 h before the bronchoscopy. All study medications were taken with at least 100 ml of water. Subjects were required to fast for 90 min before and after each dose. Only the study drug was received during therapy. The experimental protocol was approved by the local Hospital Ethical Committee, and all patients gave informed consent prior to the procedure.

BAL procedure. Fiber-optic bronchoscopy with BAL was performed at 10:00 a.m., 2 h after dose 10 of roxithromycin had been administered. The patients were premedicated with 5 to 10 mg of diazepam and were given oxygen throughout the bronchoscopy procedure. After routine inspection of the respiratory tract and aspiration of tracheobronchial secretions, the bronchoscope tip was wedged into a subsegmental bronchus of the right middle lobe. Lavage was performed by infusing a 50-ml aliquot of sterile 0.9% saline through the aspiration port and collecting it via the same port into a plastic trap by using wall suction. A total of four 50-ml aliquots (200 ml) were used, the liquid recovered after the first aliquot was discarded, and the remaining lavage fluid was pooled. The time required to perform the BAL procedure did not exceed 2 min for any patient. Blood samples were drawn just prior to administration of the 8:00 a.m. roxithromycin dose on day 6 and at the end of the bronchoscopy procedure. Plasma was promptly separated and frozen at -70°C until assayed.

Prior to any centrifugation and cell washing procedures, a portion of the BAL fluid was analyzed for cell number by hemacytometer and cell differential by using cytocentrifuge preparations (Cytospin; Shandon Southern Instruments, Sewickley, Pa.) stained with the Wright-Giemsa stain (9). Neutrophils, macrophages, and lymphocytes were recorded

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TABLE 1. Estimation of volume of ELF recovered by lavage with urea as marker of dilution for eight patients with mild chronic bronchitis

Patient no.	Urea (mg/100 ml) in:		ELF vol (ml)	Albumin (mg/ml) in:	
	BAL cells	ELF		Plasma	ELF
1	0.99	17.9	2.76	38.6	2.7
2	1.38	18.9	3.15	40.2	1.8
3	0.6	36.3	1.42	30.9	2.2
4	0.42	30.8	1.62	35.8	4.9
5	0.51	28.9	1.57	35	3.6
6	0.33	22.3	1.28	33.4	3.6
7	1.37	24	3.9	44.6	2.0
8	0.53	41.5	1.23	42.4	3.1
Mean \pm SD	0.8 \pm 0.4	27.6 \pm 8.4	2.1 \pm 1.0	37.6 \pm 4.7	3.0 \pm 1.0

as the percentage of total leukocytes, excluding epithelial cells. Bronchial epithelial cells were counted as the percentage of total cells. Lavage fluid samples were then centrifuged at 4°C (5 min, 800 \times g) to separate cells from the fluid components. Cell pellets and fluid supernatant fluids were stored at -70°C until analyzed.

Use of urea to determine the volume of ELF recovered by BAL. To quantify the apparent volume of epithelial lining fluid (ELF) obtained by BAL, we used urea as an endogenous marker of ELF dilution, as described by Rennard et al. (16). Since urea diffuses readily through the body, the urea concentration in plasma and the in situ urea concentration in ELF are identical (20, 21). In this context, 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: volume of ELF (milliliters) = total amount of urea in lavage fluid recovered (milligrams)/concentration of urea in plasma (milligrams per milliliter). Once the volume of ELF recovered is known, then any acellular component (e.g., albumin or roxithromycin) can be referenced to the volume of ELF from which it was obtained. The urea content of lavage fluid samples was determined by using a commercially available kit (65-UV; Sigma Chemical Co., St. Louis, Mo.), as previously described (16). The albumin concentrations in BAL fluid and in plasma were determined by enzyme-linked immunoassay (17).

Roxithromycin assay. Roxithromycin concentrations in plasma, BAL cells, and BAL supernatant fluid were determined by using a high-pressure liquid chromatography technique with electrochemical detection (M. A. Lefebvre, A. Mignot, L. Millerioux, J. P. Akbaraly, and J. B. Fourtillan, Abstr. Pittsburgh Conf. Exposition, abstr. no. 118, 1986). The assay sensitivity was 0.2 μ g/ml, and the intraassay and interassay coefficients of variation were <10%. Linear regression analysis of the standard calibration lines yielded a correlation coefficient of >0.99, indicating excellent linearity of the assay between 0.2 and 20.0 μ g/ml. Prior to the roxithromycin assay, cell pellets were lysed by sonication, BAL supernatants were concentrated in a freeze-dryer, and the powder was suspended in methanol to yield a 25-fold concentration over the original volume. The intracellular concentration of the antibiotic was calculated from micrograms of antibiotic per 10⁶ cells and expressed as micrograms per milliliter of cell. In determining the antibiotic intracellular concentration, two assumptions were made: (i) cells recovered by BAL consisted only of alveolar macrophages, and (ii) the mean volume of 10⁶ human alveolar macrophages was 2.5 \times 10⁻³ ml (4).

Statistical evaluation. All data are presented as mean \pm

standard deviation. Statistical values including results noted as falling below the lower limit of detection by the assay were calculated by using the lowest detectable value obtained for that assay. A *P* value of 0.05 or less was considered significant.

RESULTS

Characterization of the cell population and volume of ELF recovered by BAL. The volume recovered by BAL was 83 \pm 26 ml, with a mean total number of cells of (402 \pm 207) \times 10³/ml of lavage fluid. The differential cell count revealed 89 \pm 9% macrophages, 7 \pm 12% lymphocytes, and 4 \pm 3% neutrophils. The percentage of bronchial epithelial cells was <2% in all patients except two, in whom it was 4 and 6%, respectively. Erythrocyte contamination of the cell pellets was not observed except in one patient (patient 8), in whom erythrocytes were recorded as less than 0.1% of the total BAL cells, corresponding to a contamination of BAL fluid by less than 3 μ l of whole blood. On the basis of the measurements of the total amount of urea in the lavage fluid recovered by a 150-ml lavage and the concentration of urea in plasma, the calculated volume of ELF recovered averaged 2.1 \pm 1.0 ml (Table 1). The measured albumin concentration in lavage fluid was 85 \pm 46 μ g/ml. When urea was used as a marker of ELF dilution, albumin concentration in ELF was calculated to be 3.0 \pm 1.0 mg/ml. This value represented 8.1 \pm 3.2% of the albumin concentration in plasma in the same individuals.

Pulmonary disposition of roxithromycin. Roxithromycin concentrations in blood, ELF of the lower respiratory tract, and cells recovered by BAL are given in Table 2. The mean predose concentration in plasma prior to dose 10 of roxithromycin was 8.1 \pm 4.0 μ g/ml. At 2 h after drug administration, a time approximating the time to maximum concentration in plasma (*T*_{max}), the mean concentration was 11.4 \pm 5.7 μ g/ml. The mean level of roxithromycin in ELF (2.0 \pm 1.7 μ g/ml) was 17% of the simultaneous levels in plasma (11.4 \pm 5.7 μ g/ml). In two patients, although the drug was detected in BAL fluid, it could not be quantified accurately because the levels were below the threshold of sensitivity of the assay method. With the lowest detectable value obtained for that assay for these two patients, there was a significant relationship (*r* = 0.75; *P* < 0.02) between roxithromycin levels in plasma and ELF (Fig. 1). It was also noted that the concentration of roxithromycin in ELF was directly related to the concentration of albumin in ELF (*r* = 0.74; *P* < 0.05). The concentration of the antibiotic in cells recovered by BAL (21 \pm 10 μ g/ml) was 2 and 10 times higher than in plasma (11.4 \pm 5.7 μ g/ml) and ELF (2.0 \pm 1.7 μ g/ml), respectively.

TABLE 2. Pulmonary disposition of roxithromycin in eight patients with mild chronic bronchitis

Patient no.	Roxithromycin concn ($\mu\text{g/ml}$) in:			
	Plasma (predose on day 6)	Plasma (during bronchoscopy)	ELF	BAL cells
1	5.1	7.4	2.2	31.8
2	3.3	3.8	<0.2	31.8
3	6.0	8.9	<0.2	12.1
4	10.7	15.5	4.7	11.3
5	5.1	6.0	0.5	11.8
6	13.6	20.1	3.2	22.3
7	13.3	15.2	1.7	32.7
8	7.6	14.1	3.3	13
Mean \pm SD	8.1 \pm 4.0	11.4 \pm 5.7	2.0 \pm 1.7	21.0 \pm 10.0

DISCUSSION

Most previous studies of antibiotic penetration into the respiratory tracts of humans have been based only on comparisons of antibiotic levels in blood and sputum (3), and it is not known how well these agents enter the lung acinus, the site of the pneumonic process. In the present study the pulmonary disposition of roxithromycin, a new macrolide antibiotic potentially active against most pulmonary pathogens including *L. pneumophila* (1, 10, 11, 19), was directly assessed by BAL, a procedure whereby the cells and fluid lining the alveolar epithelial surface can be harvested in a high degree of purity (9, 18).

The BAL technique is based on the concept that aliquots of sterile, physiological saline infused through the bronchoscope mix with the ELF, and therefore when the saline is recovered by aspiration the cellular and molecular components of the ELF are recovered along with it. Because the recovered fluids are a variable mixture of saline, ELF, and ELF components, it has, however, been difficult to estimate the actual concentration of recovered molecules in the ELF in situ. In the present study, urea was used as an endogenous marker of ELF dilution as described by Rennard et al. (16), taking advantage of the fact that urea, a plasma component that is easily measured, diffuses freely throughout the body including the alveolar wall (20, 21). With this approach, the total volume of recovered ELF can be calculated by simple dilution principles, and once the volume of ELF recovered is known, it is possible to estimate the actual concentration of molecules in ELF in situ and thus determine whether the

concentration of the various molecules is relevant to pharmacological activity or biological function. The major disadvantage of using urea as a marker is that some urea from sources other than the recovered ELF is likely to diffuse into the recovered lavage fluid during the lavage procedure. Thus the ELF volume determined by the urea method probably represents an overestimate of the true ELF volume. However, even though the volume of ELF estimated by urea is an apparent volume, several lines of evidence suggest that this value is a reasonably accurate measure of the actual volume of ELF recovered (16). First, with a single instillation and immediate aspiration, it is likely that a minimum of 80% of the recovered urea derives from the urea present in situ in ELF prior to the lavage procedure (16). Second, the value of the albumin concentration in ELF, 8.1% that in plasma when calculated from ELF volume, is in good agreement with experimental animal studies that have directly measured the albumin concentration in ELF (15; W. M. Mentz, M. R. Knowles, J. B. Brown, J. T. Gatzky, and R. C. Boucher, *Am. Rev. Respir. Dis.* 129:A315, 1984). Third, the calculated total lung ELF volume obtained by combining the estimate of the ELF volume recovered by lavage with an estimate of the fraction of lung lavaged by a standard method is consistent with the estimates of total lung ELF volume by morphometric techniques (16).

In the present study, we found that increasing levels of roxithromycin in the blood resulted in an increase in the pulmonary ELF levels, with a correlation whose slope (0.2) suggested that a 5:1 ratio between blood and lung ELF levels exists. Accordingly, if the level of roxithromycin in blood and the MIC of roxithromycin for the infecting organism are known, a reasonable estimate of the ratio of antibiotic in situ concentration to MIC can be made. Roxithromycin in human plasma is highly and specifically bound to alpha 1-acid glycoprotein, with a free fraction of ca. 13% when the drug is studied alone at a concentration in plasma of 8.4 $\mu\text{g/ml}$ (R. Zini, M. P. Tournet, J. Barre, D. Tremblay, and J. P. Tillement, *Abstr. Roxithromycin Int. Congr.*, abstr. no 36, 1987). 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 low as 13% of the plasma concentration, since the free fraction alone is able to penetrate within the ELF. In fact, as demonstrated by the present study and previous work (2, 15, 16), 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 ELF was 8% of the level in plasma, a value very similar to that reported by Rennard et al. (16) and others (7, 15). Although the alpha 1-acid glycoprotein con-

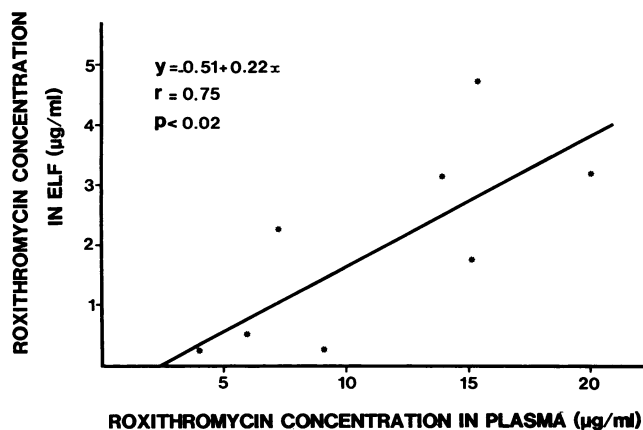


FIG. 1. Roxithromycin concentration in ELF as a function of roxithromycin concentration in plasma.

centration in ELF was not measured in our study, it seems likely that the ratio of alpha 1-acid glycoprotein concentrations in ELF and plasma was very similar to or slightly higher than the ratio of albumin concentrations in ELF and plasma, since their molecular masses are in the same range (alpha 1-acid glycoprotein, 44,000 daltons; albumin, 68,000 daltons). Indeed, Bell et al. demonstrated that the respective distribution coefficients of alpha 1-acid glycoprotein and albumin between BAL effluents and matched serum samples from normal healthy nonsmokers and smokers were 1.6 and 1.1, further confirming that their concentrations in ELF would vary in the same range in case of modification of the alveolar capillary membrane protein permeability (2). These findings may explain why roxithromycin levels in ELF were 20% of those in plasma and not 13%, as expected if no proteins were present in ELF, and also why there was a significant relationship between albumin and roxithromycin concentrations in ELF.

When administered perorally, roxithromycin achieved a pulmonary intracellular (intraphagocytic) concentration ($21 \pm 10 \mu\text{g/ml}$) of 2- and 10-fold greater than those in plasma ($11.4 \pm 5.7 \mu\text{g/ml}$) and ELF ($2.0 \pm 1.7 \mu\text{g/ml}$), respectively. Thus despite low extracellular concentrations in ELF, roxithromycin was markedly accumulated by resident human alveolar macrophages in concentrations largely exceeding the MICs of the drug for 90% of the *L. pneumophila* strains (MIC, $0.25 \mu\text{g/ml}$) (11, 19). These results are in keeping with previous studies, conducted mainly in vitro on radiolabeled compounds, which indicated that macrolides (erythromycin, spiramycin, and roxithromycin) and lincosamides can be concentrated by certain mammalian cells (5, 13, 14; M. B. Carlier, A. Zenebergh, and P. M. Tulkens, 26th ICAAC, abstr. no. 929, 1986). Cellular accumulation of these drugs is compatible with an active energy-requiring membrane transport process, as demonstrated by dependence upon cell viability, physiologic environmental temperature, and mitochondrial oxidative respiration (5, 6, 14). These facts may explain why, in our study, concentrations of roxithromycin in plasma and BAL cells were not correlated.

Entry of antimicrobial agents into phagocytes (especially macrophages) is obviously a prerequisite if these drugs are to inactivate viable intracellular organisms. On the other hand, documenting uptake of a given antibiotic by phagocytes does not prove that the drug will have antibacterial activity within the cells. Nevertheless, it is of interest that erythromycin and rifampin, two antibiotics which are markedly accumulated by phagocytes, are able to inhibit the multiplication of *L. pneumophila* within human monocytes (8) and are effective in animal models of infection and in human legionellosis (12).

In summary, the present study has shown that when roxithromycin is administered perorally, its disposition includes distribution to the pulmonary ELF of the lower respiratory tract in a concentration dependent on blood levels and alveolar capillary membrane protein permeability. It has also shown that this antibiotic is markedly accumulated by resident alveolar macrophages in concentrations largely exceeding the MBC of the drug for *L. pneumophila* and that sampling both fluid and cells lining the alveolar acini offers a suitable method for studying pulmonary disposition of antibiotics in humans.

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