The penetration of erythromycin into human bronchial mucosa

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1 In this paper the pharmacokinetic relation between concentrations of erythromycin in plasma and in bronchial mucosa was established.

2 Sixteen patients undergoing bronchoscopy received 1 g erythromycin by continuous infusion in 30 min.

3 Two bronchial mucosal samples were taken from each patient sometime after drug administration. Concentrations were determined in the mucosal sample and in several plasma samples collected at different times before the mucosal sample.

4 Rate constants for the distribution between plasma and tissue were calculated on the basis of the assumption of passive diffusion of the free drug.

5 Mean values of the distribution rate constants, calculated by a nonlinear curve fitting procedure, were 2.40 h^{-1} for distribution from plasma to tissue and 1.28 h^{-1} for that from tissue to plasma.

6 These values correspond to a half-life for the distribution of the free drug of 33 min and a free fraction of the drug in mucosal tissue of 0.20.

Keywords erythromycin bronchial mucosa

Introduction

Erythromycin is an antibiotic with activity against Gram-positive micro-organisms, *Legionella* and *Mycoplasma*. Therefore it is suitable for treatment of lower respiratory tract infections. Since the drug must obviously reach the site of infection, i.e. the bronchial mucosa and the bronchial secretions, dosage schedules should take into account the pharmacokinetic relations between plasma concentrations and those at the site of infection. The problems in assessing this kind of relation for antibiotics have been reviewed recently (Smith & LeFrock, 1983). As far as erythromycin is concerned, published human data refer mainly to concentrations in bronchial secretions (Bergogne-Berezin *et al.*, 1978; Gerding *et al.*, 1986). However, these concentrations may differ from those in bronchial mucosa (Pennington, 1981). The present study was undertaken to determine how the course of

the erythromycin concentration in bronchial mucosa is related to that in plasma. For this purpose the rates of diffusion of erythromycin from plasma to bronchial mucosa and *vice versa* were established in patients undergoing bronchoscopy. The pharmacokinetic approach chosen made it possible to estimate average diffusion rate constants from only one mucosal sample taken in duplicate from each patient.

Methods

Patients

Eighteen patients undergoing bronchoscopy for diagnostic reasons entered the study. The reasons for bronchoscopy were not relevant to the study. Patients were informed of the aim of the study,

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its experimental character and the procedure before informed consent was given. The hospital Review Committee had no objections to the study.

Experimental procedure

All patients received 1 g of erythromycin as the lactobionate (Abott N. V., Amstelveen, The Netherlands) by a continuous intravenous infusion of 30 min duration. Blood samples were collected in heparinized tubes 0, 10, 30 and 60 min after discontinuation of the infusion and then at hourly intervals until the mucosal specimens were taken during bronchoscopy. Biopsies were taken from the carinal part of two different segmental bronchi.

Antibiotic assay

Blood samples were centrifuged for 10 min at 1500 g. The antibiotic content of the plasma was measured by an agar diffusion bio-assay procedure (Mattie *et al.*, 1973), using 20 ml Antibiotic Medium no. 1 (Difco Laboratories, Detroit, Michigan) (pH 8.0) for the bottom layer and 10 ml of the same medium, seeded with a 1:100 dilution of an 18 h culture of *Sarcina lutea* ATCC 9341, as the top layer. Plasma concentrations were measured against a standard in pooled human plasma.

For the assay of the tissue content the specimens were placed immediately after bronchoscopy in plastic tubes and weighed (range 2 to 40 mg). Then 150 μ l of phosphate buffered saline were added. After shaking for 30 s in a Vortex mixer the tissue sample was homogenized by ultrasound cell destruction at an amplitude of 6 μ for 3 min on ice (ultrasonic disintegrator, 100 Watt model, Measuring & Scientific Equipment Ltd, London, Great Britain). In preliminary experiments this method had given reproducible results with complete recovery when tissue was incubated with erythromycin or when erythromycin was added to the homogenate. Concentrations in the homogenate were measured as described for plasma, but against a standard in saline. All assays were performed on the day of sampling. The lowest measurable concentration was 0.06 mg l⁻¹. The error in the assay was less than 10%.

Pharmacokinetic analysis

For pharmacokinetic analysis of the results the following model equation (Kunst & Mattie, 1978) was used:

$$dC_T/dt = k_p C_p - k_T C_T \quad (1)$$

In this equation C_T and C_p are the mean tissue concentration and plasma concentration, respectively, and k_p and k_T are rate constants of distribution. The constant k_T represents the rate constant for the distribution of the free drug between plasma and tissue, if it is assumed that diffusion of the drug between plasma water and extracellular tissue water is passive, according to Fick's law. Equation 1 can thus be modified as follows:

$$dC_{u,T}/dt = k_T(C_{u,p} - C_{u,T}) \quad (2)$$

in which $C_{u,T}$ and $C_{u,p}$ are unbound tissue and plasma concentrations, respectively. The constant k_p in equation 1 is therefore a hybrid constant, consisting of the diffusion rate constant k_T , the percentage plasma protein binding and the free tissue concentration expressed as a fraction of the total tissue concentration ($f_{u,T}$). This is given by the equation

$$k_p = f_{u,p} \times k_T / f_{u,T} \quad (3)$$

in which $f_{u,p}$ is the non-protein-bound fraction of the drug in plasma. It is assumed that $f_{u,T}$ is not concentration dependent.

The pharmacokinetic model chosen to describe the course of the plasma concentration during and after continuous infusion was a two-compartment open model. According to this model the plasma concentration after the infusion period is given by the equation

$$C_p = C_1 e^{-\lambda_1 t} + C_2 e^{-\lambda_2 t} \quad (4)$$

After substituting equation 4 into equation 1 integration leads to the following equation:

$$C_T = k_p \left\{ \frac{C_1}{k_T - \lambda_1} (e^{-\lambda_1 t} - e^{-k_T t}) + \frac{C_2}{k_T - \lambda_2} (e^{-\lambda_2 t} - e^{-k_T t}) \right\} + C_{T\tau} e^{-k_T t} \quad (5)$$

in which $C_{T\tau}$ is the tissue content at the end of the infusion period.

The equation for the plasma concentrations during continuous infusion is as follows

$$C_p = \frac{1 - e^{-\lambda_1 \tau}}{1 - e^{-\lambda_1 \tau}} C_1 + \frac{1 - e^{-\lambda_2 \tau}}{1 - e^{-\lambda_2 \tau}} C_2 \quad (6)$$

in which t' is the time lapse since the start of the infusion and τ the total duration of the infusion.

After substituting equation 6 into equation 1 integration leads to the following equation for $C_{T\tau}$:

$$C_T = k_p \left\{ \frac{C_1}{1-e^{-\lambda_1\tau}} \left(\frac{1-e^{-k_T\tau}}{k_T} - \frac{e^{-\lambda_1\tau}-e^{-k_T\tau}}{k_T-\lambda_1} \right) + \frac{C_2}{1-e^{-\lambda_2\tau}} \left(\frac{1-e^{-k_T\tau}}{k_T} - \frac{e^{-\lambda_2\tau}-e^{-k_T\tau}}{k_T-\lambda_2} \right) \right\} \quad (7)$$

Values for the pharmacokinetic constants of equation 4 were calculated for the individual patients according to a least squares method using the curve fitting procedure NLIN of the SAS computer program package. For four patients only three plasma samples were drawn before the mucosal sample was taken. In these cases the curve fitting procedure could not be used and the pharmacokinetic constants were estimated graphically: λ_2 was calculated from the last two concentrations; λ_1 , after subtraction, from the first concentration and an arbitrary concentration of 0.1 mg l^{-1} as the second concentration.

Substitution of these individual values into equation 5 (in which equation 7 is substituted for C_T) yields a model equation for the tissue content with two unknowns (k_p and k_T), for each patient. Using the NLIN procedure a pair of average estimates of k_p and k_T for all patients together, with their respective standard errors, could be calculated for which the sum of squares of the differences between C_T according to

equation 5 and the observed values of C_T , is minimised.

Results

Eighteen patients entered into the study. The samples from two patients contained large amounts of cartilage and therefore were not used. The results for the remaining sixteen patients are shown in Figure 1. It can be seen that for most patients the results for the two different mucosal samples were in good agreement, with a few exceptions that probably reflect inhomogeneity of the tissue. For the calculations the means of the two samples were used.

Pharmacokinetic parameters of the course of the plasma concentration were calculated for individual patients by fitting equation 4 to the observed values. Substitution of these parameters into equation 5 yields a graphical representation of the relation between the two unknowns k_p and k_T for each individual patient (Figure 2). The figure shows that 12 of the graphs converge to a common area while the graphs for four other patients are different. Therefore the results were analyzed for the cluster of 12 patients as well as for all patients together. The results are listed in Table 1. The estimates of k_p and k_T correspond

Figure 1 Concentrations of erythromycin in plasma (●) and in two samples of bronchial mucosa (○) from 16 patients after a continuous infusion of 1 g in 30 min. The equation $C_p = C_1e^{-\lambda_1t} + C_2e^{-\lambda_2t}$ was fitted to the plasma concentrations. Patients are shown from left to right in the order of the time of tissue sampling.

* Patients with deviating distribution constants (see Results)

Figure 2 Relation between rate constants of distribution of erythromycin from plasma to bronchial mucosa (k_p) and *vice versa* (k_T). Calculations in 16 patients were based on concentrations in plasma and in a sample of bronchial mucosa.

Table 1 Pharmacokinetic parameters of erythromycin in plasma and in bronchial mucosa

	12 patients	16 patients
C_1 (mg l ⁻¹)	11.7 (9.2)*	9.7 (8.7)*
λ_1 (h ⁻¹)	40.3 (39.9)*	37.7 (35.0)*
C_2 (mg l ⁻¹)	22.0 (9.8)*	21.7 (9.4)*
λ_2 (h ⁻¹)	0.63 (0.39)*	0.80 (0.50)*
k_p (h ⁻¹)	2.40 (0.37)**	2.35 (1.30)**
k_T (h ⁻¹)	1.28 (0.26)**	1.72 (1.05)**

* s.d.

** s.e. mean.

The equation $C_p = C_1 e^{-\lambda_1 t} + C_2 e^{-\lambda_2 t}$ was fitted to the plasma concentrations; k_p and k_T are rate constants for the distribution from plasma to tissue and *vice versa*, respectively. A group of 12 patients was used for a separate calculation (see **Results**).

to the area where the individual graphs in Figure 2 converge. There is little difference in the mean value of the distribution rate constant k_T calculated for the cluster of 12 patients and that found for all 16 patients, although the error is larger for the latter group. When the data for the four apparently different patients were analyzed separately k_T was found to be $1.56 (\pm 1.02) \text{ h}^{-1}$ and k_p $1.06 (\pm 0.62) \text{ h}^{-1}$. The value of k_T (12 patients), representing the rate constant for the distribution of free drug between plasma and tissue and *vice versa*, corresponds to a half-life for the distribution of 33 min.

By substituting the mean values of C_1 , C_2 , λ_1 and λ_2 for the cluster of 12 patients in equation

Figure 3 Calculated average course of concentration of erythromycin in plasma and in bronchial mucosa. Graphs were constructed with the calculated pharmacokinetic parameters of 12 patients (Table 1) for total concentration (continuous lines) and for concentration of free drug (interrupted lines). C_p and C_T are total plasma concentration and total tissue content, respectively. $C_{u,p}$ and $C_{u,T}$ are free plasma and tissue concentrations, respectively.

4 an average course of the plasma concentration was calculated. By substituting the same mean values together with the mean values of the distribution constants k_p and k_T in equation 5, an average course of the concentration in tissue could be determined. The result is shown in Figure 3. The plasma protein binding of erythromycin, determined by equilibrium dialysis, was 63% and not concentration-dependent in the range of actual concentrations. Consequently after substituting 0.37 for $f_{u,p}$ in equation 3 the free fraction in tissue ($f_{u,T}$) was calculated to be 0.20. On the basis of these values of $f_{u,p}$ and $f_{u,T}$ an estimated course of the free plasma and free tissue concentrations could be established. The result is also shown in Figure 3. The calculated average time of equilibration between plasma and bronchial mucosa is 35 min after discontinuation of the infusion and the average maximal free tissue concentration at that time is 4.6 mg l^{-1} .

Discussion

The results of this study provide a fairly precise estimate of the pharmacokinetic constants governing the relation between concentrations of erythromycin in plasma and those in bronchial mucosa. In a rather homogeneous group of 12 out of 16 patients it was found that the rate constant for distribution of the free drug is 1.28 h^{-1} and that the free fraction of the total tissue

content is 0.20. In a subgroup of four patients the rate constant for distribution of the free drug (1.56 h^{-1}) was not very different, but the calculated value of k_p was. One can speculate why this is so, but from equation 3 it appears that differences in both plasma binding and tissue binding may play a role. Although the rate constants were calculated after a continuous infusion of 30 min duration, they should not depend on the mode of administration. This may be regarded as an advantage over the commonly used parameter, namely the ratio between total tissue content and total plasma concentration. Not only is this ratio time-dependent, as can be seen from the relevant equations as well as the courses calculated for the concentrations in plasma and in tissue, but it also gives an inaccurate impression of the concentration of the free drug in tissue. This is important, because it is generally agreed that in extracellular infections the actual concentration of the drug to which bacteria are exposed is the free extracellular concentration (Kunst & Mattie, 1978). In the present study this free concentration was found

to be only 20% of the total tissue concentration, obviously because of nonspecific binding to tissue.

The distribution rate constant corresponds to a rather long half life of 33 min. Because of this the maximal concentration in tissue is reached 35 min after discontinuation of the infusion and is much lower than the maximal plasma concentration. As can be seen from the high values of λ_1 found for our patients the distribution of erythromycin over a large volume also occurs much more rapidly than that between plasma and bronchial mucosa. This implies that a distribution rate constant calculated from the plasma concentration only cannot be used to estimate the rate of penetration into a particular tissue (Noe & Kumor, 1983).

As for the therapeutic consequences of these findings, it may be argued that for a bacteriostatic drug such as erythromycin the prolonged concentration in bronchial mucosa is more favourable than high peaks and a rapid decline, as suggested by the course of the plasma concentration.

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