Rifampicin concentrations in bronchial mucosa, epithelial lining fluid, alveolar macrophages and serum following a single 600 mg oral dose in patients undergoing fibre-optic bronchoscopy

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The concentrations of rifampicin in epithelial lining fluid (ELF), human alveolar macrophages (AM), bronchial mucosal biopsies and serum were measured after a single oral dose of 600 mg rifampicin from 15 patients undergoing fibre-optic bronchoscopy. The samples of ELF, AM, the bronchial mucosal biopsies and serum were obtained ∼2–5 h after dosing. The mean concentrations (mean ± standard error) were 5.25 mg/L (±0.67) in ELF, 7.93 mg/kg (±1.61) in bronchial biopsies, 15.48 mg/L (±1.41) in serum and 251.8 mg/L (±65.92) in alveolar macrophages. These site concentrations exceeded the MIC₉₀ for common respiratory pathogens including Mycobacterium tuberculosis and support the observed clinical efficacy of rifampicin in the treatment of a wide range of respiratory tract infections.

Introduction

The clinical efficacy of antimicrobial agents is not only dependent on bacterial susceptibility but also on appropriate penetration of the antibiotic to the site of infection. Rifampicin is a bactericidal drug primarily used in the treatment of tuberculosis. Although active against intracellular organisms, it is also highly bactericidal against rapidly growing extracellular organisms. For these reasons, it has gained increasing use in combination with other agents for the treatment of relatively difficult to treat staphylococcal infections.

For many antimicrobials, it is important to maintain inhibitory serum concentrations during most of the intervals between doses in order to optimize clinical and bacteriological success.¹ However, these observations have largely been validated for extracellular bacterial infections. The response of intracellular bacterial infections may be predicted better from tissue to serum ratios,²,³ especially for infected body sites possessing a permeable membrane such as the alveolar epithelium.

Early studies of drug distribution in the respiratory tract examined whole lung tissue, sputum and pleural fluid. However, this can result in unsatisfactory information. Antimicrobial concentrations in whole lung represent an average of several different tissue compartments. Sputum concentrations often vary because of sampling difficulties, sputum pooling and contamination with blood, while drug instability makes conclusions about pharmacokinetic behaviour unreliable. Pleural fluid concentrations are only likely to be useful in predicting efficacy in the treatment of empyema. Sampling the bronchial mucosa has been used to indicate the likely response to infections, such as exacerbations of chronic bronchitis and possibly bronchiectasis, while bronchoalveolar lavage (BAL) has provided the opportunity to study alveolar infections such as pneumonia. Epithelial lining fluid (ELF) and alveolar macrophages (AM) represent extracellular and intracellular sites, respectively, and have been increasingly studied as predictors of drug behaviour. The objective of our study was to determine the distribution of rifampicin in pulmonary tissues in comparison with those in serum after a standard single adult oral dose of 600 mg in order to define optimal concentrations better when treating intracellular or extracellular lung infection.

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Materials and methods

Patients

Fifteen patients (11 males and four females) undergoing routine fibre-optic bronchoscopy for diagnostic purposes were included in the study. The study was approved by the Hospital Ethics Committee and all patients gave written informed consent before entry into the study. The mean age was 63.7 years (range 34–75 years) and the mean weight was 65 kg (range 48–91 kg). Patients were excluded if they had an active pulmonary infection, known hypersensitivity or intolerance to rifampicin, or evidence or history of significant pre-existing haematological, renal, cardiovascular or hepatic disease. Exclusion criteria also included: disturbance of hepatic function (serum transaminase more than twice the upper limit of normal and/or bilirubin above the normal range); other medications that could potentially interact with rifampicin; patients with malabsorption or other conditions affecting drug uptake; smoking on a regular basis within 6 months of study entry; lactation or pregnancy. Subjects were not permitted to take any drugs other than those prescribed by a physician in the 2 weeks prior to the study.

Dosage regimens

This was an open-label pharmacokinetic study designed to assess the distribution of rifampicin in bronchial tissues and fluids following the administration of a single oral dose of 600 mg (2 × 300 mg capsules) administered with 120 mL of tap water. The exact time of administration was noted.

Sample collection

Each patient underwent standard bronchoscopic examination between 2 and 5 h post-dose (having fasted for the previous 4 h). BAL was performed on each patient using 200 mL of pre-warmed 0.9% saline divided into four 50 mL aliquots, and 0.1 mg/L. Human serum calibrators and internal controls were prepared in human plasma at concentrations of 0.03–1 mg/L. A phosphate buffer and 0.9% sodium chloride calibrators and internal controls were prepared in sterile distilled water.

Concentrations of rifampicin (Lepetit, Spain) were measured in macrophages, ELF and plasma samples using an adaptation of a microbiological assay using Iso-sensitest agar (Oxoid, Basingstoke, UK) supplemented with 30 g/L potassium dihydrogen orthophosphate (Scientific and General, West Midlands, UK) to give a final pH of 5.5. One per cent Bacteriological agar (Oxoid) was added to aid media setting. The assay organism (Staphylococcus aureus ATCC 29219) was grown overnight at 35–37°C in Tryptone soya broth (Oxoid) and diluted in sterile distilled water to give an optical density of 0.022 at 630 nm before surface flooding the plate. Five millimetre diameter wells were punched into the agar using a random pattern and the plugs removed and discarded. The agar plates were then stored at 4°C until use. The lower limit of detection of the assay was 0.03 mg/L and the between-assay coefficient of variation was 6.8% over a concentration range of 0.03–1 mg/L.

Calibrators and internal controls were prepared in human serum (Tissue Culture Services, Botolph Clayton, UK), for the assay of plasma samples, or pH 6 phosphate buffer for the assay of macrophages, BAL and bronchial mucosa, and 0.9% sodium chloride for any BAL samples requiring concentration. Calibrators used, irrespective of the matrix, were 1.28, 0.64, 0.32, 0.16 and 0.08 mg/L with internal controls of 1.0 and 0.1 mg/L. Human serum calibrators and internal controls were prepared prior to the study, aliquots were made in Sarstedt pots (Sarstedt, Germany) and stored at –70°C until use (thawed once and then discarded). Phosphate buffer and 0.9% sodium chloride calibrators and internal controls were prepared on the day of assay. Quality assurance samples were prepared on the day of assay in human plasma at concentrations within the calibrator range. The samples were tested in triplicate using a random pattern. The wells were filled to give a convex meniscus. Plates were incubated overnight at 37°C and zone diameters measured using an image analyser (Imaging Associates, Thame, UK), which was pre-
programmed with Bennett’s calculation to give a line of best fit. Urea concentrations in BAL were determined using a commercial diagnostic kit (Sigma BUN endpoint; Sigma, Poole, UK). The urea assay measured blood urea nitrogen (Kit No. UV-66). This was modified by adjusting the sample: reagent ratio, which was adjusted to 1:1 instead of the 1:200 ratio recommended when testing serum samples because of the extremely low concentrations of urea in ELF. Standards were prepared from a single quality control reference standard and diluted in normal saline. The between-assay coefficient of variation was 7.8%.

**Calculation of antibiotic concentration**

Calculations of the antibiotic concentrations in biopsy, AM, BAL and plasma were calculated as described previously. Briefly, for bronchial biopsy,

\[
\text{[assay concentration (mg/L) × (weight of sample + volume of buffer)/weight of sample (mg) = concentration (mg/kg)}
\]

For epithelial lining fluid, a urea dilution method was used as described by Rennard et al. The method was applied to the standard lavage (BAL) specimen and the antibiotic concentration was calculated as follows:

\[
(AC × BU)/LU = ELF \text{ concentration (mg/L)}
\]

where \(AC\) = antibiotic concentration in lavage (mg/L), \(BU\) = blood urea level (mmol/L) and \(LU\) = lavage urea (mmol/L).

Macrophage concentrations were determined using the mean cell volume of an alveolar macrophage of 2.48 µL/10⁶ cells from data derived by Johnson et al. The cellular antimicrobial concentration was calculated as follows: (i) total cell count (TCC) = volume of lavage × cell count/mL × fraction of AM in count; (ii) total cell volume (TCV) = TCC × volume of each cell; and (iii) cell antimicrobial concentration = (assayed concentration × volume of resuspended pellet)/TCV.

**Results**

BAL was performed safely in all 15 patients enrolled in the study. Of these, three were excluded due to technical difficulties; two BAL samples were contaminated with blood and one sample had a very low alveolar macrophage count (<1 × 10⁶). No adverse events that were possibly drug related were recorded. Following bronchoscopy two subjects experienced self-limited light-headedness of unclear aetiology during the period of observation.

Mean concentrations of rifampicin in serum, ELF, macrophages and biopsies of bronchial mucosa, as well as site:serum ratios, are presented in Table 1. However, this ratio does not take into account the kinetics of tissue distribution, which is best assessed by determination of the ratios of the area under the curve (AUC) values. Both peak tissue levels and the AUC in tissue may provide important information regarding efficacy. However, determination of the AUC in tissue requires serial samples that were not possible in our study population. Figure 1 shows the concentrations of rifampicin measured in serum, ELF, AM and bronchial mucosa plotted against time following administration of the drug in relation to the MIC₉₀₉ for *Mycobacterium tuberculosis*.

**Table 1. Concentrations in serum and pulmonary compartments after a single dose of rifampicin 600 mg administered 2–5 h (mean 3.45 h) before sampling**

<table>
<thead>
<tr>
<th>Site</th>
<th>Range</th>
<th>Mean concentration (S.E.)</th>
<th>Mean site:serum ratio (mean of individual data)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum (mg/L)</td>
<td>8.9–23.4</td>
<td>15.5 (1.41)</td>
<td>–</td>
</tr>
<tr>
<td>ELF (mg/L)</td>
<td>3.3–7.5</td>
<td>5.3 (0.67)</td>
<td>0.34</td>
</tr>
<tr>
<td>AM (mg/L)</td>
<td>145.4–738.7</td>
<td>251.8 (65.92)</td>
<td>16.26</td>
</tr>
<tr>
<td>BM (mg/kg)</td>
<td>6.2–16.6</td>
<td>7.9 (1.61)</td>
<td>0.51</td>
</tr>
</tbody>
</table>

**Discussion**

Despite more than 40 years of clinical use, surprisingly little is known about the distribution of rifampicin in human lung tissue. However, the high concentration of rifampicin in AM was in agreement with previously published observations, which have shown that rifampicin penetrates well into most tissues. Concentrations of rifampicin in human liver, stomach and bone usually exceed simultaneous serum levels because of its lipid solubility. Alveolar macrophage drug concentration:plasma drug concentration ratios were approximately 16:1 (Table 1). Based upon the reported range of MICs of rifampicin for *M. tuberculosis*, this finding would indicate that considerable antimicrobial activity resides within AM. For example, for most *M. tuberculosis* strains the MIC is 0.5 mg/L, inhibitory ratios of 15:1 to 25:1 are present in AM, which is adequate for most strains of *M. tuberculosis*. The *in vivo* effect of high AM concentrations on antimicrobial activity and the clinical significance of plasma, bronchial mucosa or ELF rifampicin concentrations are unknown. In general, high inhibitory or killing ratios are viewed as favourable in the treatment of infectious diseases, especially those affecting the lung. It is likely that the high intrapulmonary drug concentrations observed in this study contribute to the effectiveness of rifampicin in the treatment of pulmonary tuberculosis and other infections.

The alveolar epithelial membrane presents a significant barrier by separating ELF from blood and should be considered a pharmacologically protected environment. Clinical efficacy, particularly against pathogens confined to sites
separated from blood by natural barriers, may be more closely related to site/serum-specific drug concentrations. This study has shown satisfactory concentrations of rifampicin at all potential sites of respiratory infection in relation to known MIC₉₀ of target pathogens, and compares favourably with data on other rifamycin derivatives. Although the levels for ELF are lower than those for bronchial mucosa and serum concentrations, they are still well in excess of the MIC₉₀ of rifampicin for *S. aureus* (0.02 mg/L), *Legionella* spp. (0.25 mg/L) and *M. tuberculosis* (0.5 mg/L), which is indicated in Figure 1.

Based on these single dose observations in uninfected volunteers, current dosage regimens of an oral dose of 600 mg rifampicin would appear to be pharmacokinetically appropriate for target pathogens. The limitation of single dose studies is recognized. Ideally multiple-dose steady-state information is desirable and is a logical sequence to this study. Because of the fact that rifampicin is a potent inducer of its own metabolism, we expect that concentrations at steady state will usually be lower than those following single dose administration. Although technically challenging it would be valuable to repeat these studies in patients undergoing active treatment for lung infection in order to provide more clinically applicable information.

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**References**


Rifampicin concentrations in patients undergoing bronchoscopy


