

Azithromycin concentrations at the sites of pulmonary infection

D.R. Baldwin, R. Wise, J.M. Andrews, J.P. Ashby, D. Honeybourne

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ABSTRACT: Azithromycin is a new macrolide antimicrobial. The distribution to the potential sites of pulmonary infection was assessed after the administration of a single 500 mg oral dose to 22 patients undergoing fiberoptic bronchoscopy. Concentrations of azithromycin in sputum, bronchial mucosa, epithelial lining fluid (ELF) and alveolar macrophages (AM) were determined at intervals up to 96 h after dosing. The mean serum concentration was low at 12 h ($0.13 \mu\text{g}\cdot\text{ml}^{-1}$, SEM 0.05) but was still detectable at 96 h ($0.01 \mu\text{g}\cdot\text{ml}^{-1}$). In contrast, peak sputum ELF, bronchial mucosal and AM levels were found at 48 h. Bronchial mucosal concentrations were significantly greater than ELF concentrations, which were in turn greater than sputum concentrations. Mean peak AM concentrations were sixfold greater than bronchial mucosal concentrations ($23 \mu\text{g}\cdot\text{ml}^{-1}$, SEM 5.1 and $3.89 \mu\text{g}\cdot\text{ml}^{-1}$, SEM 1.2, respectively). The high intracellular concentrations indicate that azithromycin is likely to be effective for sensitive intracellular pathogens and the favourable penetration into sputum, ELF and bronchial mucosa suggest that it should be useful in pneumonia and bronchial infections. *Eur Respir J.*, 1990, 3, 886-890.

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The increasing range of antimicrobial agents necessitates the development of improved methods to predict their clinical efficacy and to elucidate any advantages over existing agents. *In vitro* study of the antimicrobial activity of drugs is well established, but more recently the value of measuring activity of an antimicrobial agent at the potential site of infection has been appreciated [1, 2]. Sputum and bronchial mucosa have been used to represent sites of infections such as occur in bronchitis and exacerbations of bronchiectasis [3, 4], and pneumonia has been represented by constituents of bronchoalveolar lavage (BAL) fluid, namely the alveolar macrophage (AM) and epithelial lining fluid (ELF) [5, 6].

Azithromycin has enhanced *in vitro* activity against *Haemophilus influenzae* compared to erythromycin and has a particularly long tissue half-life [7]. It has been shown to be concentrated in alveolar macrophages and polymorphs [8, 9] but *in vivo* studies in humans have not been performed.

Important methodological factors have made the interpretation of antimicrobial concentrations in the sputum, AM and ELF difficult [10, 11]. In this study the concentrations of azithromycin, a new macrolide agent, have been measured in the potential sites of infection-sputum, AM and ELF and compared with the serum levels.

Patients and methods

Twenty two patients were studied, (6 female) who were undergoing fiberoptic bronchoscopy for diagnostic purposes. They were all given a single 500 mg oral dose of azithromycin and were divided into five groups according to sampling time after the dose (12, 24, 48, 72 and 96 h). There were four patients in each group, except for the 72 h group where there were six, and bronchoalveolar lavage (BAL) was performed in only four of these. Patients were excluded if they had, on biochemical and clinical evaluation, evidence of significant cardiac, renal or hepatic impairment. The smoking history of patients was recorded as it is known that this may affect AM antimicrobial concentrations [12]. There were two smokers in each group except in the 72 h group, where there were three, and the 96 h group, where there was only one.

All patients provided informed written consent and the study was approved by the Hospital Ethical Committee.

Sample collection

Fiberoptic bronchoscopy was performed using a premedication of 160 mg of nebulized 4% lignocaine

and 0.6 mg of intramuscular atropine, and sedation with 3–7 mg of intravenous midazolam. After careful examination of the airways, bronchial secretions were gently aspirated. Following this "microlavage" was performed in a distal lung sub-segment (see below). Then a standard 200 ml bronchoalveolar lavage was performed in a different lung segment (right middle lobe or lingula) with gentle aspiration following each 50 ml aliquot. The recovered fluid from the first 50 ml was processed separately. Bronchial biopsies were then taken from macroscopically normal subcarinal mucosa (absence of erythema, induration or nodularity). Lastly a serum sample was taken for azithromycin, total protein and urea concentration and a red cell count (RCC).

"Microlavage"

Before the technique described below was used in patients, a short experiment on post mortem lungs was performed. Normal post mortem lungs were obtained and the right middle lobe and lingular segmental orifices identified. The segments were then inflated with air using a tightly fitting tube with syringe and three way tap attached. A standard bronchial brush tube was then gently passed along a middle lobe or lingular orifice until wedged. Methylene blue dye was injected, the pleural surface observed for staining and the time necessary to aspirate back a small sample noted. We found that the pleural surface was stained after the injection of 10–15 ml and the time taken to obtain the sample only 20 s. Larger volumes did not improve yield because dye was forced back proximal to the point at which the tube was wedged.

The technique was then employed in 28 patients (using 20 ml of warmed normal saline instead of dye) and the cell differential count of the aspirate compared with that of conventional BAL. The volume of ELF recovered by conventional lavage was estimated by calculating the ELF total protein concentration using the microlavage sample (see below). The differential counts for microlavage and conventional BAL showed good correlation (fig. 1). No epithelial cells were noted in the microlavage sample, adding further evidence that distal ELF was sampled. The ELF volume recovered by conventional BAL was less applying microlavage than when applying the urea dilution method directly.

Calculation of epithelial lining fluid volume

A urea dilution method was used described by RENNARD *et al.* [10]. This method was applied to the microlavage specimen, where the lavage dwell time was short enough for the urea to accurately represent that contained in the epithelial lining fluid (ELF) [11]. The concentration of total protein in the ELF was then determined as follows:

Microlavage:

$$\text{ELF volume} = \frac{\{\text{urea}\} \text{ lavage} \times \text{volume lavage}}{\{\text{urea}\} \text{ ELF}^*}$$

$$\{\text{protein}\} \text{ ELF} = \frac{\text{vol lavage} \times \{\text{protein}\} \text{ lavage}}{\text{ELF volume}}$$

$$* = \{\text{urea}\} \text{ blood}$$

The ELF protein concentration was then applied to the standard BAL to calculate the volume of ELF recovered, as BAL protein varies far less with lavage dwell time [11].

The median percentage contribution of ELF to lavage volume was 1.17% (range 0.18–3.33%) applying microlavage and was 1.51% (range 0.53–4.06%) using the direct method (95.4% confidence interval for difference = 0.11 to 0.71; Wilcoxon).

Sample processing

Bronchial secretions were diluted 1:2 with cold phosphate buffer (pH 8.0). The microlavage and standard BAL specimens were centrifuged immediately in the bronchoscopy suite, at 400 g for 5 min, after a small aliquot had been removed for the total and differential cell counts. Total cell count preparations were again made immediately, and to avoid cell loss due to adherence to surfaces, siliconized glassware and teflon containers were used at all times. The BAL supernatant was freeze dried and then resuspended in distilled water to yield a tenfold concentration. Bronchial biopsies were collected into a humidity chamber and any blood stained samples were discarded. Following addition of a known volume of cold phosphate buffer (pH 8.0) both the cell component, bronchial secretion and biopsies were ultrasonicated on ice for 2 min at 50% duty cycle (W225 Sonicator Heat Systems Ultrasonics Inc.).

The total white and red cell counts were performed using a Neubauer chamber. Samples were discarded if the RCC was greater than $4 \times 10^5 \text{ ml}^{-1}$. The differential count was performed on cellular monolayers prepared in triplicate by cytocentrifugation of 10^5 cells ($100 \mu\text{l}$ of 10^6 white cells ml^{-1}) at 450 rpm for 15 min (Shandon cytospin II). Slides were air-dried for 30 min prior to staining with May-Grünwald-Giemsa stain.

All samples were assayed for azithromycin concentration using a microbiological plate assay in which the indicator strain *Sarcina lutea* (DRH strain Z114) was inoculated on to pre-poured plates of antibiotic media No 1 adjusted to pH 8.5 (Oxoid, Basingstoke, UK). Plates were incubated at 37°C for 18 h. Standards were prepared in phosphate buffer (pH 8.0) for bronchial secretions, cell and biopsy samples, in 9% saline for the concentrated supernatant and in 100% human serum for serum samples. The between assay coefficient of variation was 9.5% and the lower limit of sensitivity $0.01 \mu\text{g} \cdot \text{ml}^{-1}$. The BAL supernatant and microlavage sample were also assayed for total

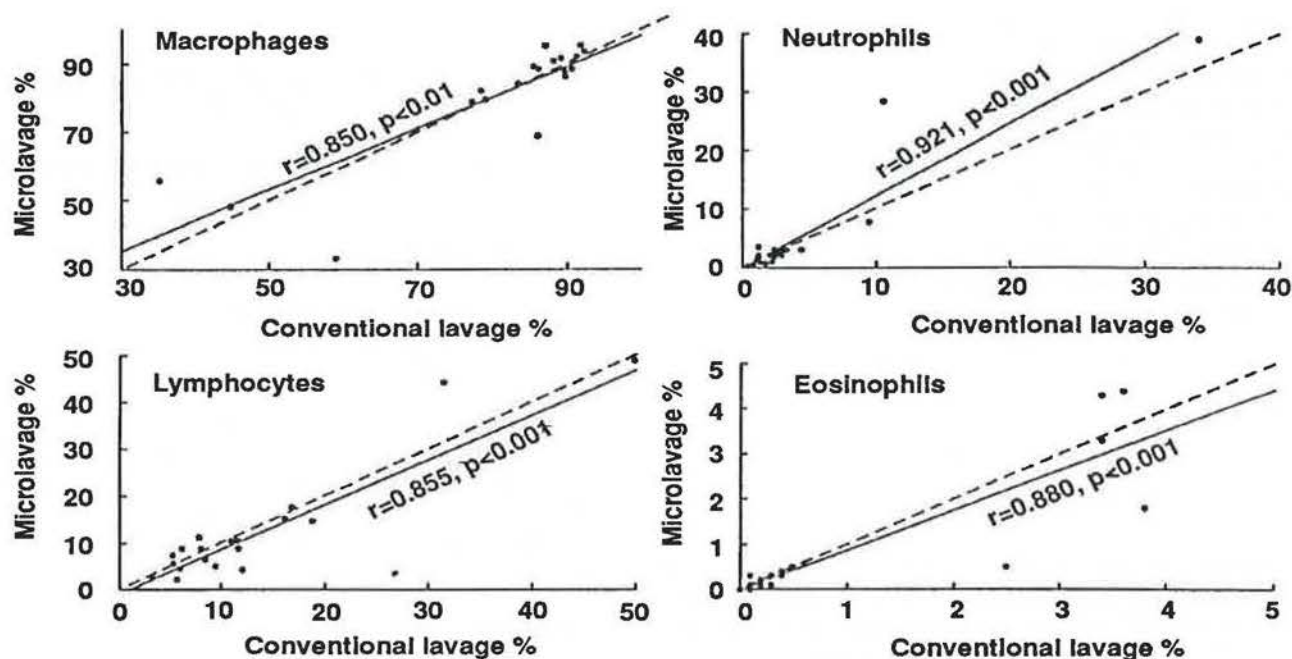


Fig. 1. – Correlation of differential cell counts — : line of equivalence.

Table 1. – Total protein and urea assays

| | Total protein | Urea |
|----------------------------|----------------|---------------------|
| Kit | Sigma - 06 | Sigma UV - 66 |
| Method | Coomassie blue | Blood urea nitrogen |
| Sample volume | 200 μ l | 500 μ l |
| Reagent volume | 900 μ l | 500 μ l |
| Coefficient of variation % | 5.1 | 4.8 |

protein and urea concentrations using modified Sigma diagnostic kits (Sigma Chemicals, Poole, UK) (table 1).

Determination of AM volume

Velocity gradient centrifugation [13] was used to determine cell volume. This was found to agree with previous morphological studies [14] and with manual measurement of cell diameters employing microscopy of a hanging droplet. Using micropipettes to measure volume of displacement produced a 100% overestimate of cell volume and was not used. The mean cell volume was $2.42 \mu\text{l} \cdot 10^6$ cells (SEM 0.41).

Results

Figure 2 shows the mean azithromycin concentrations for each sample divided according to the five sampling times. Serum concentrations were low at 12 h ($0.13 \mu\text{g} \cdot \text{ml}^{-1}$, SD 0.05) but persisted at 96 h at the lower limit of sensitivity of the assay ($0.01 \mu\text{g} \cdot \text{ml}^{-1}$).

In all other samples peak concentrations were found after 48 h. The mean peak sputum and ELF concentrations were 1.56 and $2.18 \mu\text{g} \cdot \text{ml}^{-1}$, respectively, ($p=0.259$, paired t-test). ELF concentrations were significantly higher than sputum concentration applying the paired t-test to all 22 observations (mean ELF level $1.43 \mu\text{g} \cdot \text{ml}^{-1}$, mean sputum level $0.82 \mu\text{g} \cdot \text{ml}^{-1}$; $p=0.012$). The mean peak bronchial mucosal concentration was in turn higher than ELF concentrations at $3.89 \mu\text{g} \cdot \text{ml}^{-1}$ (SEM 1.2) and there was a highly significant difference between all 22 bronchial mucosal and ELF concentrations ($p<0.001$; paired t-test).

Alveolar macrophage concentrations of azithromycin were high (mean peak $23.0 \mu\text{g} \cdot \text{ml}^{-1}$, SEM 5.1) and persisted at high levels at 96 h ($15.8 \mu\text{g} \cdot \text{ml}^{-1}$, SEM 1.1).

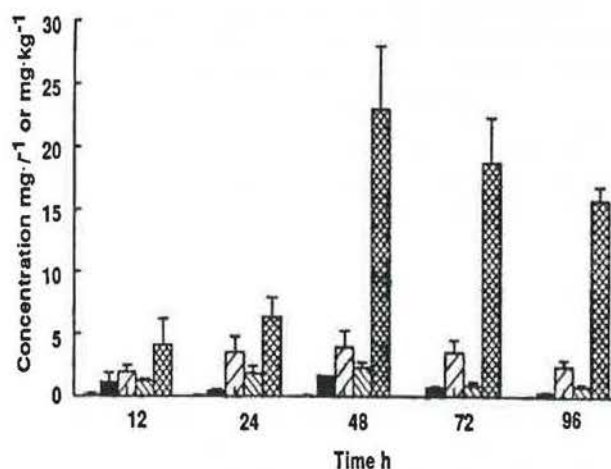


Fig. 2. – Azithromycin concentrations in the lung. ■ : serum; ▨ : sputum; ▩ : mucosa; ▤ : lining fluid; ▦ : macrophage; — : denotes SEM.

No disturbance in liver function tests was noted in any patients and no adverse reactions encountered.

Discussion

The relatively recent practice of measuring the concentrations of antimicrobial agents achieved at the site of infection has served to illuminate differences in the penetration of drugs into tissues. Studies of the penetration of different drugs into the lung [1, 2] have shown that small, lipophilic, non-protein-bound drugs tend to travel into tissues more easily. Studies undertaken *in vitro* using peripheral blood monocytes [15] or neutrophils [16], or with alveolar macrophages [17] have shown that in addition there may be active transport mechanisms involved. The measurement of antimicrobial concentrations in sputum or bronchial secretions aspirated at bronchoscopy is difficult and may be unreliable. This is because sputum has pooled in the lung over some hours and may also be contaminated with blood and saliva [2]. Lastly, the stability of some antimicrobials in sputum may vary due to pH changes and enzymatic action [18]. However, despite all these difficulties it may still be helpful to measure sputum concentrations as certainly some organisms may persist in sputum and cause damage without actually invading the mucosa [19]. In acute bronchial infections there will be adherence of bacteria to bronchial mucosa [20] and, therefore, bronchial mucosal antimicrobial concentrations are particularly relevant, and indeed may be a better predictor of clinical response [21].

In vivo studies of alveolar macrophage and ELF antimicrobial concentrations have been plagued by two principle difficulties. Firstly, when alveolar macrophages are allowed to take up antibiotics such as macrolides or quinolones *in vitro*, and are then placed in antibiotic free media, they lose most of their intracellular antibiotic very quickly, often within 10 min. This situation is analogous to the situation in BAL. Thus, before performing studies *in vivo*, it is essential to determine the efflux rate following removal of extracellular antibiotic for each drug. The likely error can then be calculated. For azithromycin loss of antibiotic from phagocytes and macrophages is very slow [9]. We have also ensured that there was no delay in separating the AM from the supernatant by centrifuging the sample immediately.

The principle difficulty with the measurement of ELF antimicrobial concentrations is the determination of ELF volume recovered in lavage fluid. Previously, workers have used a urea dilution method [10] to calculate this but this has led to overestimates of ELF volume due to movement of urea into the lavage fluid during prolonged dwell times [11]. The microlavage technique lavages a distal subsegment of the lung within 20 s, thus circumventing the problem of prolonged dwell time. The microlavage tube is only 1.7 mm in diameter and since respiratory epithelium lines airways of less than 2 mm diameter it is not surprising that this procedure gives similar differential counts to conventional BAL. We can

expect, therefore, that the ELF recovered by microlavage is representative of that recovered by conventional BAL when the aspirate from the first 50 ml is discarded.

The distribution of azithromycin in different sites within the lung serves to illustrate how different serum concentrations may be from those at the sites of infection. It is important to assess the *in vivo* tissue concentrations of drugs, such as azithromycin, with low serum concentrations and high volumes of distribution. Applying our data to the *in vitro* susceptibility of the relevant pathogens to azithromycin (table 2) [7], we consider that the drug is likely to be very effective for intracellular pathogens such as *Mycoplasma* and *Legionella spp.*, and that the high concentrations in ELF, sputum and bronchial mucosa would favour its use in pneumonia and infective exacerbations of chronic bronchitis. However, despite these high concentrations, it is important for there to be adequate serum concentrations in the event of septicaemia. Thus a *b.d.* dosage would be advisable, given intravenously for severe infections.

Table 2. — *In vitro* activity of azithromycin to respiratory pathogens

| Organism | MIC ₉₀ µg·ml ⁻¹ |
|---------------------------------|---------------------------------------|
| <i>Streptococcus pneumoniae</i> | 0.12 |
| <i>Haemophilus influenzae</i> | 1 |
| <i>Moraxella catarrhalis</i> | 0.5 |
| <i>Staphylococcus aureus</i> | 1 |
| <i>Legionella pneumophila</i> | 0.12–0.25 |

MIC: minimal inhibitory concentration.

The effectiveness of drugs with low serum but high tissue concentrations requires further study, and comparisons with other macrolides such as clarithromycin and erythromycin would be advisable.

The results of the present study may provide an insight into the process of movement of antibiotics which are taken up by phagocytic cells into tissues. The concentration of azithromycin in alveolar macrophages was found to be much higher at 48 h than 12 or 24 h ($p=0.014$, $p=0.02$ Student's *t*-test, respectively) and thereafter to slowly decrease (fig. 2). A possible explanation for this is that the initial high concentrations are a result of penetration of azithromycin from the blood to the ELF where alveolar macrophages take up the antibiotic, and that the much higher concentrations at 48 h and beyond are due to migration of a population of monocytes into the lung, which were present in the blood at the time when peak serum concentrations occurred. Work is in progress to confirm or refute this hypothesis. The alternative explanation is that the uptake is very slow and takes 48 h to reach a peak level. However, *in vitro* experiments suggest uptake takes only 24 h [9].

Azithromycin should be a useful agent, particularly where intracellular pathogens are concerned and shows good penetration into sputum, ELF, AM and bronchial mucosa.

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Concentrations d'azithromycine aux sièges de l'infection pulmonaire. D.R. Baldwin, R. Wise, J.M. Andrews, J.P. Ashby, D. Honeybourne.

RÉSUMÉ: L'azithromycine est un nouvel antimicrobien macrolide, dont la distribution aux sites potentiels d'infection pulmonaire a été appréciée après administration d'une dose orale unique de 500 mg à 22 patients subissant une fibro-bronchoscopie. Des concentrations d'azithromycine dans les crachats, dans la muqueuse bronchique, dans le liquide de revêtement épithélial, et dans les macrophages alvéolaires, ont été déterminées à des intervalles allant jusqu'à 96 h. après l'administration. La concentration sérique moyenne était faible à 12 h. ($0.13 \mu\text{g}\cdot\text{ml}^{-1}$, SEM 0.05), mais était encore détectable à 96 h. ($0.01 \mu\text{g}\cdot\text{ml}^{-1}$). Par contre, les niveaux de pointe dans l'expectoration, dans le liquide de revêtement épithélial, dans la muqueuse bronchique, et dans les macrophages alvéolaires, ont été décelés après 48 h. Les concentrations dans la muqueuse bronchique étaient significativement plus élevées que celles observées dans le liquide de revêtement épithélial qui, de leur côté, étaient supérieures aux concentrations sputaires. Les concentrations de pointe moyennes dans les macrophages alvéolaires étaient six fois plus élevées que celles de la muqueuse bronchique ($23 \mu\text{g}\cdot\text{ml}^{-1}$, SEM 5.1 et $3.89 \mu\text{g}\cdot\text{ml}^{-1}$, SEM 1.2, respectivement). Les concentrations élevées intra-cellulaires indiquent que l'azithromycine est susceptible d'être efficace sur des pathogènes sensibles et intra-cellulaires; sa pénétration favorable dans les crachats, dans le liquide de revêtement épithélial et dans la muqueuse bronchique, suggère qu'elle devrait être utile dans la pneumonie et dans les infections bronchiques.

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