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Concentrations of moxifloxacin in serum and pulmonary compartments following a single 400 mg oral dose in patients undergoing fibre-optic bronchoscopy

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The concentrations of moxifloxacin achieved after a single 400 mg dose were measured in serum, epithelial lining fluid (ELF), alveolar macrophages (AM) and bronchial mucosa (BM). Concentrations were determined using a microbiological assay. Nineteen patients undergoing fibre-optic bronchoscopy were studied. Mean serum, ELF, AM and BM concentrations at 2.2, 12 and 24 h were as follows: 2.2 h: 3.2 mg/L, 20.7 mg/L, 56.7 mg/L, 5.4 mg/kg; 12 h: 1.1 mg/L, 5.9 mg/L, 54.1 mg/L, 2.0 mg/kg; 24 h: 0.5 mg/L, 3.6 mg/L, 35.9 mg/L, 1.1 mg/kg, respectively. These concentrations exceed the MIC_{90}s for common respiratory pathogens such as *Streptococcus pneumoniae* (0.25 mg/L), *Haemophilus influenzae* (0.03 mg/L), *Moraxella catarrhalis* (0.12 mg/L), *Chlamydia pneumoniae* (0.12 mg/L) and *Mycoplasma pneumoniae* (0.12 mg/L) and indicate that moxifloxacin should be effective in the treatment of community-acquired, lower respiratory tract infections.

Introduction

Moxifloxacin (Bay 12-8039, Bayer AG, Wuppertal, Germany) is a new fluoroquinolone with a broad spectrum of antimicrobial activity. Its activity encompasses Gram-positive, Gram-negative and anaerobic bacteria as well as 'atypical' organisms. In-vitro studies have demonstrated superior activity against penicillin-sensitive, penicillin-resistant and macrolide-resistant pneumococci compared with a number of quinolones.[1] The aim of this study was to assess the concentrations of moxifloxacin in pulmonary tissues, in comparison with those in serum after a single 400 mg oral dose. A microbiological assay, previously validated by Bayer, was used to determine levels of moxifloxacin. Adequate drug penetration of potential sites of infection in the respiratory tract, especially epithelial lining fluid and alveolar macrophages[2] is important in the achievement of therapeutic efficacy. Quinolone antibiotics have been shown previously to penetrate into lung tissues more efficiently than β-lactams.[3]

Materials and methods

Twenty-two patients (17 males, five females) undergoing diagnostic fibre-optic bronchoscopy were enrolled. Three (all male) subsequently withdrew before bronchoscopy. All patients were over 18 years of age and all female subjects were post-menopausal. This study was approved by the Hospital Ethics Committee and all subjects gave fully informed, written consent. Patients were excluded if they had an active respiratory tract infection, known hypersensitivity to quinolones, significant hepatic or renal disease, or severe cardiac failure. All subjects were assessed within the 14 days before bronchoscopy. The assessment included a medical history, physical examination and blood analysis for haematology and biochemistry. There were no specifications regarding the administration of the drug relative to meals. Patients enrolled were stratified into one of three treatment groups: Group A, moxifloxacin 400 mg 2 $\pm$ 1 h before bronchoscopy; Group B, moxifloxacin 400 mg 12 $\pm$ 1 h before bronchoscopy; Group C, moxifloxacin 400 mg 24 $\pm$ 1 h before bronchoscopy. The timing of doses, in relation to food, was not specified.

Sample collection and processing

Samples of bronchial mucosa (BM) were taken from macroscopically normal areas of the lung, in addition to diagnostic samples. Standard bronchoalveolar lavage (BAL) was performed using 200 mL of prewarmed 0.9%

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saline divided into four 50 mL aliquots, followed by gentle aspiration. Aspirate from the first 50 mL was discarded to avoid contamination of the sample with larger airway fluids and cells. The remaining three aspirates were pooled and divided into two Teflon-coated containers for analysis. A small volume of lavage fluid was removed from each sample and the number of macrophages was counted, using an improved Neubauer counting chamber. The remaining lavage aspirate was immediately centrifuged at 400**g** for 5 min and the supernatant and cells separated. Approximately 2 mL of the supernatant was removed for estimation of urea content. The remaining fluid was used to measure the concentration of moxifloxacin present, by freeze drying followed by the addition of distilled water to reconstitute to one-tenth of the original volume. Other samples were prepared for assay as follows. Cell pellets were ultrasonicated on ice using a known volume of chilled phosphate buffer pH 7 before assay. BM biopsies from each patient were pooled in a humidity chamber, to avoid loss of moisture from tissue before weighing. Samples were weighed (heavily blood-stained tissue was discarded) and ultrasonicated as previously described.[3] Immediately after bronchoscopy, serum samples were taken for measurement of urea and moxifloxacin levels. All samples were stored at 4°C and protected from light before assay. All assays were performed within 2 h of collection, with the exception of those on the lavage samples.

Microbiological assay

Concentrations of moxifloxacin were measured using a microbiological assay. Briefly, assay plates (Mast Diagnostics, Bootle, UK) containing IsoSensitest agar (Oxoid, Basingstoke, UK) were flooded with an organism suspension (*Escherichia coli* 4004, Bayer Wuppertal, AG) adjusted to an optical density of 0.004 at 630 nm. Antibiotic standards, prepared in human serum (range 0.125–2 mg/L), phosphate buffer pH 7 (range 0.06–1 mg/L) and 9% sodium chloride (range 0.06–1 mg/L), internal controls and tests were applied to the plate (in triplicate following a random pattern), by filling 5 mm wells which had been cut from the agar with a cork borer. After overnight incubation at 30°C, zones were measured using an image analyser (Imaging Associates, Theme, UK) and the concentration calculated using Bennet's calculation.[4]

Calculation of antibiotic concentrations

Bronchial mucosa (BM). Moxifloxacin concentration was calculated from the formula described below.

$$\frac{\text{Assayed concentration (mg/L)} \times (\text{diluent volume} + \text{sample volume})}{\text{weight (mg)}} = \text{antibiotic concentration (mg/kg)}$$

Alveolar macrophages (AM). Antibiotic concentration in macrophages was determined assuming a mean cell volume of an alveolar macrophage of 2.48 $\mu L/10^6$ cells.[5]

Epithelial lining fluid (ELF). BAL fluid urea concentration was determined using a modified Sigma Diagnostic Kit (UV-66, Sigma Chemicals, Poole, UK). The ELF moxifloxacin level was calculated using the method described by Renard *et al.*[6]

$$\text{ACL} \times \text{BU} = \frac{\text{ELF antibiotic concentration (mg/L)}}{\text{LU}}$$

where ACL is the antibiotic concentration in the lavage fluid (mg/L), BU is the blood urea concentration (mmol/L) and LU is the lavage fluid urea concentration (mmol/L).

Results

The lower limit of quantification of the assay was 0.03 mg/L and the between-assay coefficient of variation was 8% over a concentration range of 0.04 to 1.5 mg/L. Mean concentrations of moxifloxacin in serum, ELF, macrophages and bronchial mucosa biopsies as well as mean site:serum ratios are documented in Table I. Antibiotic concentrations at all sites for each subject are given against time since dosing. It can be seen that the highest mean serum, ELF, macrophage and biopsy concentrations (3.2 mg/L, 20.7 mg/L, 56.7 mg/L and 5.4 mg/kg, respectively) occurred in group A (2.2 h post dose). The mean areas under the concentration/time curves (AUC) for 0–24 h were calculated for each site and the areas under the inhibitory curve (AUIC) were also calculated using published MIC_{90} values (Table II). None of the patients experienced severe adverse events.

Discussion

This study has demonstrated that clinically significant concentrations of moxifloxacin are achieved in serum and the respiratory tract, at all potential sites of infection, for up to 24 h post dose. Mean serum, AM, ELF and bronchial mucosa concentrations in all three groups of patients were found to exceed, by at least two-fold, the MIC_{90}s of moxifloxacin for common respiratory pathogens (MIC_{90}s for *S.pneumoniae*,[7] *M.catarrhalis*,[7] *H. influenzae*,[7] *C. pneumoniae*[7] and *M.pneumoniae*[8] are 0.25 mg/L, 0.12 mg/L, 0.03 mg/L, 0.12 mg/L and 0.12 mg/L, respectively). It is important to note that the MIC_{90}s of earlier quinolones for the pneumococcus are higher (2 mg/L in the case of ciprofloxacin).

Compared with trovafloxacin,[9] mean site:serum ratios (at 12 and 24 h) were greater for moxifloxacin. Higher site:serum ratios have been reported after multiple dosing with quinolones.[9] The AUC/MIC ratio (AUIC) indicates the relationship between the pharmacokinetics of an antibiotic

Table I. Summary of the geometric mean moxifloxacin concentrations (S.D.) in serum, epithelial lining fluid (ELF), alveolar macrophages (AM) and bronchial biopsies (BM)

Group	Site	Mean concentration (mg/L or mg/kg) (S.D.)	Mean site/serum ratio (mean of individual data) (S.D.)
A			
(mean time after last dose = 2.2 h)			
	Serum	3.22 (1.25)	
	ELF	20.7 (1.92)	6.78 (2.29)
	AM	56.7 (1.61)	18.59 (1.87)
	BM	5.36 (1.29)	1.67 (1.18)
B			
(mean time after last dose = 11.8 h)			
	Serum	1.14 (1.42)	
	ELF	5.90 (2.20)	5.19 (1.90)
	AM	54.1 (3.06)	44.61 (2.70)
	BM	1.97 (1.33)	1.74 (1.10)
C			
(mean time after last dose = 24.1 h)			
	Serum	0.51 (1.19)	
	ELF	3.57 (1.58)	6.95 (1.43)
	AM	35.9 (1.71)	70.04 (1.58)
	BM	1.06 (1.19)	2.07 (1.19)

Table II. Mean areas under the curves (AUC) and areas under the inhibitory curves (AUIC) relating to serum and pulmonary sites

		AUIC		
	AUC (mg/L/h)	*S. pneumoniae* (MIC_{90} = 0.25 mg/L)	*H. influenzae* (MIC_{90} = 0.03 mg/L)	*M. catarrhalis* (MIC_{90} = 0.12 mg/L)
Serum	36.5	145	1210	302
Bronchial mucosa	53.8	215	1793	448
Epithelial lining fluid	189	756	6300	1575
Alveolar macrophages	496	1984	16533	4133

and its pharmacodynamic interaction with pulmonary pathogens. The AUIC has been used as a surrogate marker for predicting clinical outcomes. A threshold value of 125 for the AUIC has been proposed as a marker for efficacy.[10] As shown in Table II this threshold was exceeded in all sites at the time of measurement.

Alveolar macrophage penetration by moxifloxacin was, as with other quinolones, excellent, suggesting clinical efficacy against 'atypical' organisms associated with lower respiratory tract infection such as mycoplasma and chlamydia.

Overall, moxifloxacin should be effective against a wide variety of respiratory tract pathogens. In view of recently increasing penicillin resistance of the pneumococcus, it is of importance that moxifloxacin should demonstrate efficacy against this pathogen.

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