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

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The concentrations of gatifloxacin achieved after a single 400 mg oral dose were measured in plasma, epithelial lining fluid (ELF), alveolar macrophages (AMs) and bronchial mucosa (BM) using a microbiological assay. Fourteen patients undergoing fibre-optic bronchoscopy were studied. Mean plasma, ELF, AMs and BM concentrations, respectively, at 2, 4 and 12 h were as follows: 2 h: 3.96 mg/L, 6.00 mg/L, 69.10 mg/L, 6.24 mg/kg; 4 h: 3.22 mg/L, 6.16 mg/L, 77.32 mg/L, 5.32 mg/kg; 12 h: 1.74 mg/L, 2.98 mg/L, 61.95 mg/L, 3.00 mg/kg. These concentrations exceed the MIC\textsubscript{90}s for common respiratory pathogens such as \textit{Streptococcus pneumoniae} (0.5 mg/L), \textit{Haemophilus influenzae} (0.013 mg/L), \textit{Moraxella catarrhalis} (0.05 mg/L), \textit{Chlamydia pneumoniae} (0.125 mg/L) and \textit{Mycoplasma pneumoniae} (0.06 mg/L).

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Introduction

Gatifloxacin is a new 8-methoxy-fluoroquinolone with a broad spectrum of antimicrobial activity that encompasses Gram-positive, Gram-negative and anaerobic bacteria as well as ‘atypical’ organisms. Compared with a number of other quinolones, \textit{in vitro} studies have demonstrated superior activity of gatifloxacin against lower respiratory tract pathogens such as \textit{Streptococcus pneumoniae} \textsuperscript{1} (including penicillin-resistant pneumococci), \textit{Haemophilus influenzae} \textsuperscript{2} and \textit{Moraxella catarrhalis}.\textsuperscript{3} Epithelial lining fluid (ELF), which bathes the alveoli, is an important site of infection in pneumonia.

Alveolar macrophages (AMs) represent an important site for intracellular infection.\textsuperscript{4} AMs may take up antimicrobials from the ELF or from the plasma before their migration to the alveolar spaces. The intracellular concentration of gatifloxacin is of interest in view of the intracellular characteristics of certain respiratory pathogens such as \textit{Mycoplasma pneumoniae} and \textit{Chlamydia pneumoniae}. Adequate drug penetration of such sites of infection is important in the achievement of therapeutic efficiency. The aim of this study was to measure the concentrations of gatifloxacin in pulmonary tissues, in comparison with those in plasma after a single 400 mg dose.

Materials and methods

An open-label, randomized, single-dose study was performed on patients scheduled to undergo diagnostic flexible bronchoscopy. A total of 21 patients was planned to be enrolled, with patients 1–18 randomly allocated to time points 1.5–2.5 h, 3.5–4.5 h and 11.5–12.5 h after study drug administration. Subjects 19–21 were planned to have bronchoscopy at 23.5–24.5 h after study drug administration. The numbers of subjects chosen to be included at these time points were based upon the authors’ previous experience with similar studies of antibiotic lung penetration.\textsuperscript{5} However, owing to slow recruitment the study was stopped after 16 patients (14 males) had been enrolled. One patient (a male) subsequently withdrew before bronchoscopy. All patients were over 18 years of age and both female subjects were post-menopausal. The hospital ethics committee approved this study 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 14 days before bronchoscopy. The assessment included a medical history, physical examination and blood analysis for haematology and biochemistry. Patients enrolled were...
stratified into one of three treatment groups: group A, gatifloxacin (Grunenthal GmbH, Aachen, Germany) 400 mg 2 h before bronchoscopy; group B, gatifloxacin 400 mg 4 h before bronchoscopy; and group C, gatifloxacin 400 mg 12 h before bronchoscopy.

Sample collections and processing

Bronchoalveolar lavage (BAL) was performed using 200 mL of prewarmed 0.9% 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 the 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 centrifuged immediately (in the endoscopy suite) at 400g for 5 min and the supernatant and cells were separated without delay. Approximately 2 mL of the supernatant was removed for estimation of urea content. The remaining fluid was freeze-dried and then reconstituted to one-tenth of the original volume in distilled water. This was assayed for gatifloxacin.

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. Samples of bronchial mucosa (BM) were taken from macroscopically normal areas of the lung, in addition to diagnostic samples. 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 described previously. Immediately after bronchoscopy, plasma samples were taken for measurement of urea and gatifloxacin levels. All samples were stored at 4°C protected from light and assays were performed within 1 h of collection, with the exception of those on the lavage supernatant.

Microbiological assay

Concentrations of gatifloxacin 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 AG, Wuppertal, Germany) adjusted to an optical density of 0.004 at 630 nm. Antibiotic calibrators (range 0.125–2 mg/L), prepared in human serum, phosphate buffer pH 7 and 9% sodium chloride, internal controls, quality assurance samples and tests were applied to the plate in triplicate following a random pattern, by filling 6 mm blotting paper discs (Whatman International Ltd, Maidstone, UK) to the surface. 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.7

Calculation of antibiotic concentrations

BM. Gatifloxacin concentration was calculated from the formula described below.

\[
\text{bronchial specimen antibiotic concentration (mg/kg) = \frac{\text{assayed concentration (mg/L) \times (diluent volume + sample volume)(L)}}{\text{bronchial specimen weight (mg)}}
\]

AMs. Antibiotic concentration in macrophages was determined assuming a mean cell volume of an alveolar macrophage of 2.48 μm³/10⁶ cells.8

\[
\text{ELF} \text{ BAL fluid urea concentration was determined using a modified Diagnostic Kit (UV-66, Sigma Chemicals, Poole, UK). The assay was linear over the range 0.01–0.09 mmol/L. The coefficient of variation of the method to measure urea in BAL was 7.8% (data not shown). The concentration of urea in plasma was measured by the clinical chemistry department at the City Hospital.}
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The ELF gatifloxacin level was calculated using the method described by Rennard et al.9

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

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

Fourteen subjects completed the study. One (male) assigned to group C experienced an epistaxis episode during bronchoscopy, preventing collection of blood-free samples.

The mean age of the 14 subjects was 59.5 years (range 36–77 years), mean weight 72.4 kg (range 56–103 kg), mean height 167 cm (range 155–178 cm) and mean serum creatinine at entry 89.4 mmol/L (range 70–115 mmol/L). Eight of the subjects were current smokers (none more than 10 cigarettes per day), two were lifetime non-smokers and four were ex-smokers (two had stopped within the 6 months before the study and the others, 5 and 11 years before).

The lower limit of quantification of the assay was 0.06 mg/L and the between-assay coefficient of variation was 7.5% and 8.2% at concentration ranges of 1.5 and 0.2 mg/L, and the within-assay variation for quality assurance samples within a concentration range of 0.13–1.9 mg/L gave an \( r^2 \) value of 0.9834. After BAL the mean volume of aspirate obtained was 37.6 mL (S.D. 18.85).
Gatifloxacin lung penetration

Mean concentrations of gatifloxacin in plasma, ELF, macrophages and BM biopsies as well as mean site:plasma ratios are documented in the Table. Antibiotic concentrations at all sites for each subject are given against time since dosing. Concentrations of gatifloxacin were generally higher (1.5- to 1.8-fold) in ELF and BM than in plasma. ELF and BM to plasma ratios were almost constant over the 12 h period after dosing. For AMs, concentrations of gatifloxacin were considerably higher (18- to 37-fold) than in plasma, and increased as a function of time. All patients tolerated the single dose of gatifloxacin and none of the patients experienced any serious adverse events.

Discussion

This study has demonstrated that potentially clinically effective concentrations of gatifloxacin are achieved in plasma and the respiratory tract, at all potential sites of infection. Cross validation of the gatifloxacin bioassay with high-performance liquid chromatography has been reported previously. No major metabolites of gatifloxacin with antimicrobial activity have been reported and hence bioassay levels accurately record serum and tissue levels of gatifloxacin. Peak plasma levels attained at 2 h post-dose in this study were similar to a previous study by our department in healthy volunteers. Mean plasma, AMs, ELF and BM concentrations in all three groups exceeded, by at least three-fold, the MIC90s of gatifloxacin for common respiratory pathogens (MIC90s for S. pneumoniae, H. influenzae, M. catarrhalis, C. pneumoniae and M. pneumoniae are 0.5, 0.013, 0.05, 0.125 and 0.06 mg/L, respectively).

These tissue and serum concentrations over the 12 h post-dose period are well in excess of the MIC90 for S. pneumoniae. There have been concerns about the serum and tissue levels of the older quinolone ciprofloxacin being near to the MIC90 of ciprofloxacin for pneumococcus (2 mg/L). Our study with gatifloxacin used only a single dose and did not measure serum and tissue levels at the 24 h time period. Once-daily dosing with gatifloxacin is anticipated, and therefore it would be of further interest to know levels 24 h post-dose and to undertake a multiple dosing study.

Serum levels of some quinolones have been shown to be reliable predictors of clinical response when used to derive Cmax/MIC90 (i.e. the peak serum concentration of the drug divided by the MIC90 for a specific pathogen) and AUIC (i.e. the area under the serum–time curve divided by the MIC90 for a specific pathogen). The latter is often expressed as the AUIC over a 24 h time period (AUIC24). However, this approach does not enable accurate prediction of tissue levels. Other factors such as the degree of protein binding may be relevant. Serum protein binding of gatifloxacin is low at c. 20% in healthy volunteers and is concentration independent.

Experimental evidence suggests that tissue levels of antibiotics such as quinolones and azalides correlate with efficacy. Alveolar macrophage penetration by gatifloxacin was excellent, suggesting clinical efficiency against typical and ‘atypical’ organisms associated with lower respiratory tract infections such as C. pneumoniae and M. pneumoniae.

The ratio of tissue to serum concentrations of gatifloxacin found in this study were higher in all three sites (BM, ELF and AM) at the 2 h time point compared with a single-dose (500 mg) study of levofloxacin. At 4 h gatifloxacin had a higher AM:serum ratio but lower ELF:serum and similar BM:serum ratios compared with levofloxacin. Comparable 12 h values for levofloxacin are unavailable; however, the AM:serum for levofloxacin at 6–8 h post-dose was considerably lower (9.6) than the

<table>
<thead>
<tr>
<th>Sampling time (hours post-dose)</th>
<th>Site</th>
<th>Mean concentrationa</th>
<th>Mean site:plasma ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 (n = 5)</td>
<td>plasma</td>
<td>3.96 (3.50–4.40)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ELF</td>
<td>6.00 (3.90–8.50)</td>
<td>1.51</td>
</tr>
<tr>
<td></td>
<td>AMs</td>
<td>69.10 (50.80–112.50)</td>
<td>17.51</td>
</tr>
<tr>
<td></td>
<td>BM</td>
<td>6.24 (4.60–8.70)</td>
<td>1.57</td>
</tr>
<tr>
<td>4 (n = 5)</td>
<td>plasma</td>
<td>3.22 (2.10–6.50)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ELF</td>
<td>6.16 (1.70–18.3)</td>
<td>1.74</td>
</tr>
<tr>
<td></td>
<td>AMs</td>
<td>77.32 (48.9–138.5)</td>
<td>25.25</td>
</tr>
<tr>
<td></td>
<td>BM</td>
<td>5.32 (3.10–9.10)</td>
<td>1.65</td>
</tr>
<tr>
<td>12 (n = 5)</td>
<td>plasma</td>
<td>1.74 (1.50–2.10)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ELF</td>
<td>2.98 (1.40–4.00)</td>
<td>1.75</td>
</tr>
<tr>
<td></td>
<td>AMs</td>
<td>61.95 (29.0–116.2)</td>
<td>36.67</td>
</tr>
<tr>
<td></td>
<td>BM</td>
<td>3.00 (2.80–3.20)</td>
<td>1.80</td>
</tr>
</tbody>
</table>

aMean concentration given as mg/L or mg/kg (range).
AM: serum of gatifloxacin at 12 h (36.7). Other results from mainly multiple dose studies have recently been reviewed.\(^5\) Overall, the ratios of ELF, AMs and BM to plasma concentrations of gatifloxacin indicate good penetration into the respiratory tract. Clinical efficacy, as suggested by these results, can only be confirmed by clinical trials.

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### References


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