Pharmacodynamics of moxifloxacin and levofloxacin against Streptococcus pneumoniae, Staphylococcus aureus, Klebsiella pneumoniae and Escherichia coli: simulation of human plasma concentrations after intravenous dosage in an in vitro kinetic model

Inga Odenholt1,2* and Otto Cars2

1Infectious Diseases Research Unit, Department of Clinical Sciences Malmö, Lunds University, S-20502 Malmö, Sweden; 2Antibiotic Research Unit, Department of Medical Sciences, Section of Infectious Diseases and Clinical Microbiology, Uppsala University, Uppsala, Sweden

Received 10 November 2005; returned 10 May 2006; revised 13 July 2006; accepted 8 August 2006

Objectives: To compare in an in vitro kinetic model the pharmacodynamics of moxifloxacin and levofloxacin with a concentration–time profile simulating the human free non-protein bound concentrations of 400 mg moxifloxacin intravenous (iv) once daily, 500 mg levofloxacin iv once daily and 750 mg levofloxacin iv once daily against strains of Streptococcus pneumoniae, Staphylococcus aureus, Klebsiella pneumoniae and Escherichia coli with variable susceptibility to fluoroquinolones.

Methods: The strains used in the study included S. pneumoniae ATCC 6306 (native strain), S. pneumoniae 19397 (double mutation; gyrA and parC), S. pneumoniae 4241 (single mutation; parC), S. aureus ATCC 13709 (native strain), S. aureus MB5 (single mutation; gyrA), E. coli M12 (single mutation; gyrA), E. coli ATCC 25922 (native strain) and K. pneumoniae ATCC 29655 (native strain). The strains were exposed to moxifloxacin and levofloxacin in an in vitro kinetic model simulating the free human serum concentration–time profile of moxifloxacin 400 mg once daily, levofloxacin 500 mg once daily and 750 mg once daily. Repeated samples were taken regularly during 24 h and viable counts were carried out.

Results and conclusions: A correlation was seen between both the area under the serum concentration curve and MIC (AUC/MIC) and the peak concentration/MIC (Cmax/MIC) versus area under the bactericidal killing curve (AUBKC) or \( \Delta \log_{10} \text{cfu/mL} \). Compiling all data, an AUC/MIC of \( \sim 100 \) and a Cmax/MIC of 10 gave a maximal bactericidal effect for both levofloxacin and moxifloxacin. In accordance with the results from others, our study indicated that a lower AUC/MIC was needed for S. pneumoniae in comparison with the Gram-negative bacteria studied. Moxifloxacin yielded higher AUC/MIC and Cmax/MIC against the investigated Gram-positive bacteria in comparison with levofloxacin 500 mg once daily and 750 mg once daily.

Keywords: fluoroquinolones, pharmacokinetics, PK/PD

Introduction

During the past decade, the integration of pharmacokinetics and pharmacodynamics has become increasingly important for determining optimal dosing schedules of antibiotics.1–6 The fluoroquinolones are characterized by a concentration-dependent bactericidal activity and the ability to induce a post-antibiotic effect against both Gram-positive and Gram-negative bacteria.7–9 The ratio between the 24 h area under the serum concentration curve and MIC (AUC/MIC) and the peak concentration/MIC (Cmax/MIC) seem to be the pharmacodynamic indices that correlate to efficacy in in vitro kinetic models, in animal studies and in humans.1–6,10–13 Several investigators have studied the activity of fluoroquinolones in in vitro kinetic models and shown that members of this drug class may differ in their pharmacodynamic properties.12,14–16

The aim of the present study was to compare the pharmacodynamics of moxifloxacin and levofloxacin against strains of Streptococcus pneumoniae, Staphylococcus aureus, Klebsiella pneumoniae and Escherichia coli with differing antibiotic
Pharmacodynamics of fluoroquinolones

susceptibility. An in vitro kinetic model was used, where the concentration–time profiles of free (non-protein bound) drug in humans obtained with 400 mg moxifloxacin intravenous (iv) once daily, 500 mg levofloxacin iv once daily and 750 mg levofloxacin iv once daily were simulated.

Materials and methods

Bacterial strains and media

The strains used in the study included S. pneumoniae ATCC 6306 (native strain), S. pneumoniae 19397 (double mutation in gyrA and parC), S. pneumoniae 4241 (single mutation in parC), S. aureus ATCC 13709 (native strain), S. aureus MB5 (single mutation in gyrA), E. coli M12 (single mutation in gyrA), E. coli ATCC 25922 (native strain) and K. pneumoniae ATCC 29655 (native strain). The mutant strains were obtained from Bayer HealthCare AG, Wuppertal, Germany, and the native strains from the Department of Microbiology, Uppsala Sweden. Before each experiment the Gram-negative strains were grown for 6–7 h at 37°C in air in Mueller–Hinton broth (Difco Laboratories, Detroit, MI, USA) supplemented with 25 mg Mg²⁺ and 50 mg Ca²⁺ yielding an inoculum of ~5 × 10⁸ cfu/mL. The Gram-positive strains were grown in Todd–Hewitt broth at 37°C in air (with 5% CO₂ for S. pneumoniae) yielding an inoculum of ~5 × 10⁸ cfu/mL. During the experiments, samples for viable counts were seeded on blood agar plates (Colombia agar base with 5% horse blood, Department of Microbiology, Uppsala Sweden). The limit of detection of the viable counts was 5 × 10³ cfu/mL.

Determination of MICs

The MICs of moxifloxacin and levofloxacin for the investigated strains were determined in Todd–Hewitt broth (Gram-positive strains) or in Mueller–Hinton broth supplemented with 50 mg Ca²⁺ and 25 mg Mg²⁺ (Gram-negative strains) in triplicate on different occasions by macro-dilution technique with an inoculum of 5 × 10⁵ cfu/mL according to the Clinical Laboratories Standards Institute (formerly NCCLS).¹⁷ The MICs were defined as the lowest concentration of the antibiotics that gave no visible growth.

Determinations of antibiotic concentrations

The concentrations of moxifloxacin and levofloxacin during the in vitro kinetic experiments were determined by a microbiological agar diffusion method, using E. coli MB 3804 as the test organism.¹⁸ The bacteria were cultured in Todd–Hewitt broth for 4–5 h. Thereafter 0.4 mL of the suspension was added to 1 L of Iso-Sensitest agar (Oxoid Ltd, Basingstoke, Hampshire, UK) and poured into plates. After the plates were dried, 0.03 mL volumes of all samples and standards diluted in Todd–Hewitt broth were applied into agar wells. The assays were made in triplicate and the plates were incubated overnight at 35°C. The limit of detection was 0.1 mg/L and the coefficient of variation on samples analysed on different days was 9%.

In vitro kinetic model

The pharmacodynamics of the antibiotics for the investigated strains was studied in an in vitro kinetic model described previously.¹⁹–²² The model consists of a spinner flask with a 0.45 µm filter membrane and a pre-filter fitted in between the upper and the bottom part in order to prevent bacterial dilution. A magnetic stirrer ensures homogeneous mixing of the culture and prevents membrane pore blockage. In one of the sidearms of the culture vessel, a silicon membrane is inserted to enable repeated sampling. The other arm is connected by thin plastic tubing to a vessel containing fresh medium. The medium is removed from the culture flask, through the filter, at a constant rate with a pump. Fresh sterile medium is sucked into the flask at the same rate by the negative pressure built up inside the culture vessel. The antibiotic was added to the vessel and eliminated at a constant rate according to the first-order kinetics \( C = C₀e^{-kt} \), where \( C₀ \) is the initial antibiotic level, \( C \) the antibiotic level at the time \( t \), \( k \) the rate of elimination and \( t \) the time elapsing since the addition of antibiotic. The apparatus was placed in a thermostatic room at 37°C during the experiments.

Determination of the antibacterial effect

Before the experiments, the culture vessel was filled with Todd–Hewitt broth or Mueller–Hinton broth and bacteria were added at a starting inoculum of ~5 × 10⁹ cfu/mL. Two dose levels of levofloxacin and one dose level of moxifloxacin, which are currently used in the clinical situation, were studied. Moxifloxacin was added at a concentration of 2.0 mg/L corresponding to the free (non-protein bound) fraction obtained after a dose of 400 mg iv and the flow rate was adjusted to give a half-life of 12 h.²¹ Levofloxacin was added at concentrations of 4 and 6 mg/L corresponding to the free (non-protein bound) fraction obtained after a dose of 500 and 750 mg iv, respectively. The simulated half-life was set to 7 h.²⁴ One sample was withdrawn at each of various times (0, 1.5, 3, 5, 7, 12 and 24 h) and if necessary diluted in phosphate-buffered saline (PBS). At least three dilutions of each sample were spread onto blood agar plates and incubated at 37°C, and the colonies were counted after 24 h. The limit of detection of the method was 5 × 10¹ cfu/mL. The experiments were performed in duplicate for each bacterial strain and concentration. The antibacterial effect was described in two ways: by calculation of the log change in viable counts between time 0 and 24 h (Alog cfu/mL), where a negative value indicates a net killing effect during the period of the experiment, and by calculation of the area under the bactericidal killing curve (AUBKC) using the log linear trapezoidal rule for the period from 0 to 24 h. A value of the AUBKC of 122 indicates a static effect, a lower value a net killing.

Results

MICs

The MICs for the investigated strains are shown in Table 1.

Antibiotic concentrations

The Cmax of moxifloxacin was 2.02 ± 0.2 mg/L. The corresponding figures for levofloxacin 500 and 750 mg were 4.37 ± 0.37 and 5.8 ± 0.35 mg/L, respectively. The mean elimination half-lives were 11 h for moxifloxacin, 7.5 h for levofloxacin 750 mg and 7 h for levofloxacin 500 mg.

Experiments

Table 2 shows the pharmacodynamic indices AUC/MIC and Cmax/MIC together with the resulting antibacterial effects expressed as Alog cfu/mL and AUBKC. A rapid bactericidal effect was obtained with all three regimens against the wild-type and single mutant strain of S. pneumoniae (Figure 1a and b) The mutant was reduced below the detection limit with all three regimens, while for the wild-type a pronounced regrowth occurred with the low levofloxacin dose. For the strain 1937 with double
mutations in both gyrA and parC, neither drug exerted any bactericidal effect (Figure 1c). However, moxifloxacin achieved a lower AUBKC in comparison with both doses of levofloxacin. This was also true for the native strain of S. aureus, where moxifloxacin gave the lowest AUBKC in comparison with levofloxacin at both dosage regimens although the strain was completely killed with both drugs at 24 h (Figure 2a). For S. aureus MB5 (single mutation in gyrA) there was regrowth with all regimens at 24 h, although less pronounced for moxifloxacin (Figure 2b). For the native strain of E. coli eradication was seen for all three regimens with the lowest AUBKC seen for moxifloxacin (Figure 3a). For the mutant strain of E. coli, initial killing was followed by regrowth with all three regimens (Figure 3b). Again moxifloxacin gave the lowest AUBKC. The native strain of K. pneumoniae was completely killed after 1 h with all regimens with similar AUBKC (data not shown).

The correlation between AUC/MIC and the antibacterial effect (compiling results from all experiments) are shown in Figure 4 (a and b). For both AUBKC and Dlog cfu/mL, a maximal effect was achieved when the AUC/MIC exceeded 24/C24 = 100. The same pharmacodynamic index seemed to apply for both drugs (Figure 5a).

Table 1. MIC values of levofloxacin and moxifloxacin for the investigated strains

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Levofloxacin (mg/L)</th>
<th>Moxifloxacin (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. pneumoniae ATCC 6306</td>
<td>1</td>
<td>0.125</td>
</tr>
<tr>
<td>S. pneumoniae 4241</td>
<td>1</td>
<td>0.25</td>
</tr>
<tr>
<td>S. pneumoniae 19397</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>S. aureus ATCC 13709</td>
<td>0.25</td>
<td>0.03</td>
</tr>
<tr>
<td>S. aureus MB5</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td>E. coli ATCC 25922</td>
<td>0.016</td>
<td>0.012</td>
</tr>
<tr>
<td>E. coli M12</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>K. pneumoniae ATCC 29655</td>
<td>0.016</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Discussion

The major pharmacodynamic indices that correlate to clinical and bacteriological efficacy of fluoroquinolones are by most researchers thought to be the ratio between the 24 h AUC/MIC and Cmax/MIC.1–6 However, the magnitude of the PK-PD index needed is still under discussion and seem to vary according to the type of fluoroquinolone bacterial species and the immune status of the patients.12,14–16 In a clinical study, including 134 patients...
Pharmacodynamics of fluoroquinolones

Figure 1. (a) The killing effect of levofloxacin 750 mg (open squares), levofloxacin 500 mg (filled squares) and moxifloxacin (filled triangles) against *S. pneumoniae* ATCC 6306; controls, open circles (mean of two experiments). (b) The killing effect of levofloxacin 750 mg (open squares), levofloxacin 500 mg (filled squares) and moxifloxacin (filled triangles) against *S. pneumoniae* 4241; controls, open circles (mean of two experiments). (c) The killing effect of levofloxacin 750 mg (open squares), levofloxacin 500 mg (filled squares) and moxifloxacin (filled triangles) against *S. pneumoniae* 19397; controls, open circles (mean of two experiments).

Figure 2. (a) The killing effect of levofloxacin 750 mg (open squares), levofloxacin 500 mg (filled squares) and moxifloxacin (filled triangles) against *S. aureus* ATCC 13709; controls, open circles (mean of two experiments). (b) The killing effect of levofloxacin 750 mg (open squares), levofloxacin 500 mg (filled squares) and moxifloxacin (filled triangles) against *S. aureus* MD5; controls, open circles (mean of two experiments).

with respiratory tract, skin and soft tissue and complicated urinary tract infections, an AUC/MIC ratio of >100 resulted in a failure rate of 1% in comparison with 11.5% with an AUC/MIC of 25–100. All failures were seen in the group of patients with respiratory tract and skin and soft tissue infections.4

In the present study, the pharmacodynamics of moxifloxacin was compared with those of levofloxacin against wild-type bacteria and bacteria with defined mutations for fluoroquinolone resistance. For both drugs, similar pharmacodynamic indices for maximal antibacterial activity were found. However, of the three dosage regimens simulated, higher values of AUC/MIC and C\text{max}/MIC were achieved for moxifloxacin against all Gram-positive bacteria and a better antibacterial effect (AUBKC) was seen for moxifloxacin against five of the eight bacterial strains.

Several studies have indicated a difference in the pharmacodynamics of fluoroquinolones between Gram-positive and Gram-negative bacteria. MacGowan et al.14 studying *S. pneumoniae* and *Pseudomonas aeruginosa* in an *in vitro* kinetic model showed that a higher AUC/MIC value was needed to clear *P. aeruginosa* in comparison with that of *S. pneumoniae*. This variability between Gram-positive and Gram-negative strains has also been described by other authors.3,4,12,15,16 In the present study, such a tendency was also shown, e.g. for levofloxacin at both dosage regimens, where *S. pneumoniae* ATCC 6306 and *S. pneumoniae* 4241 were cleared completely after 12 h in five out of six experiments, while regrowth occurred at similar or higher AUC/MIC and C\text{max}/MIC for *E. coli* M12. These results are in accordance with the findings of Schubert et al.,25 who studied *S. pneumoniae* 4241 in a slightly different *in vitro* kinetic model, and also found that levofloxacin and moxifloxacin at the free concentration corresponding to a dose of 500 mg once daily and 400 mg once daily, respectively, cleared this strain after 11 h. Klepser et al.16 suggested from the results of an *in vitro* kinetic study of different quinolones against *S. pneumoniae* that in the absence of the influence of host defense an AUC/MIC between 50 and 100 would obtain near maximal antibacterial effects (total drug). Zhanel et al.,26 also in an *in vitro* kinetic model, suggested a free AUC/MIC of 35–63 in order to prevent regrowth of multidrug-resistant *S. pneumoniae*. Other investigators have also documented bacteriological eradication of...
S. pneumoniae with respiratory fluoroquinolones with an AUC/MIC of 30–64. In the present study, the native strain of S. aureus was cleared with both levofloxacin 500 and 750 mg as well as with moxifloxacin. Low AUBKCs (<18) were seen for all three regimens. However, for S. aureus with a single mutation in gyrA, regrowth occurred at 24 h with all three regimens after an initial /C24 4 log10 reduction in cfu. The AUC/MIC was <52, Cmax/MIC /C20 4 and AUBKC >80 for all regimens. Concerning the Gram-negative strains, both native strains of E. coli and K. pneumoniae were rapidly cleared in the present study with AUC/MIC > 420 and a AUBKC of <21. However with the E. coli strain with a single mutation in gyrA, regrowth occurred with all three regimens after an initial 99.9% kill. The highest AUC/MIC ratio reached was 104 for levofloxacin 750 mg. In an in vitro pharmacodynamic model, Madaras-Kelly et al. 30 suggested a value of 100 as a breakpoint to prevent selection of resistant mutants of P. aeruginosa treated with ciprofloxacin and ofloxacin. Drusano et al. 2 showed in a animal model with lomefloxacin against P. aeruginosa that an AUC/MIC (total drug) of >120 gave a 99% survival of the animals compared with 75% with an AUC/MIC of 64. Forrest and coworkers found an AUC/MIC ratio of >125 to correlate with clinical and microbiological cure in critically ill patients with predominately Gram-negative nosocomial pneumonia. 3

In conclusion, our study showed that an AUC/MIC of ~100 and a Cmax/MIC of 10 gave a maximal bactericidal effect for levofloxacin and moxifloxacin for both endpoints (AUBKC and change in viable counts). Moxifloxacin with simulated free concentrations following a dose of 400 mg yielded higher

Figure 3. (a) The killing effect of levofloxacin 750 mg (open squares), levofloxacin 500 mg (filled squares) and moxifloxacin (filled triangles) against E. coli ATCC 25922; controls, open circles (mean of two experiments). (b) The killing effect of levofloxacin 750 mg (open squares), levofloxacin 500 mg (filled squares) and moxifloxacin (filled triangles) against E. coli M12; controls, open circles (mean of two experiments).

Figure 4. (a) The relationship between AUBKC and AUC/MIC. (b) The relationship between ∆log cfu/mL and AUC/MIC.

Figure 5. (a) The relationship between AUBKC and AUC/MIC for levofloxacin (open squares) and moxifloxacin (filled squares). (b) The relationship between AUBKC and AUC/MIC for S. pneumoniae (open squares) and Gram-negative strains (filled squares).
Pharmacodynamics of fluoroquinolones

AUC/MIC and C_{\text{peak}}/MIC against the investigated Gram-positive bacteria in comparison with levofloxacin 500 mg once daily and 750 mg once daily. In accordance with the results from others, our study indicated that a lower AUC/MIC was needed for S. pneumoniae in comparison with the Gram-negative bacteria studied. However, additional strains must be studied to determine the optimal target drug exposure. This in vitro kinetic model provides a valuable tool and a complement to animal studies to determine what target should be reached in order to maximize the antibacterial effect. However, like other in vitro kinetic models, the model more mimics the effects in an immunocompromised host, since the effects of the immune system are not taken into account.

Acknowledgements

This study was in part supported by a grant from Bayer HealthCare AG, Wuppertal, Germany.

Transparency declarations

None to declare.

References