Pharmacodynamics of moxifloxacin, levofloxacin and sparfloxacin against *Streptococcus pneumoniae*

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An *in vitro* pharmacokinetic model (IVPM) was used to simulate the human serum pharmacokinetics of moxifloxacin, levofloxacin and sparfloxacin, and to compare their pharmacodynamics against *Streptococcus pneumoniae* exhibiting a wide range of susceptibilities to fluoroquinolones. Logarithmic-phase cultures were exposed to peak concentrations achieved in human serum of moxifloxacin, levofloxacin or sparfloxacin with oral doses of 400, 500 and 200 mg, respectively. Human elimination pharmacokinetics were simulated, and viable bacterial counts were measured at 0, 1, 2, 4, 6, 8, 24 and 36 h. Moxifloxacin was rapidly bactericidal (>3 logs of killing) against all 10 *S. pneumoniae* strains, with 99.9% kills of eight strains occurring within 1–3 h after dosing. Maximum kills ranged from 5 to 6 logs. Moxifloxacin eradicated seven strains from the IVPM within 8 h of the first dose, and eradicated two other strains within 24 h. Although levofloxacin and sparfloxacin were also bactericidal against all 10 *S. pneumoniae* strains, the rates of killing were somewhat slower, with sparfloxacin exhibiting the slowest rate of kill. In summary, moxifloxacin’s increased anti-pneumococcal potency compared with levofloxacin and its more favourable pharmacokinetics compared with sparfloxacin provided enhanced pharmacodynamic activity against some *S. pneumoniae* strains when maximum doses were simulated in an IVPM.

Introduction

*Streptococcus pneumoniae* is a leading cause of pneumonia, bacteraemia, otitis media and sinusitis. Since the 1970s, β-lactam resistance and resistance to multiple classes of antibiotics have been steadily increasing worldwide, limiting the therapeutic options for treatment of some infections.

The fluoroquinolones are one class of antibacterials that are currently being developed for enhanced potency against *S. pneumoniae*. Moxifloxacin, a new 8-methoxyquinolone, is four- to eight-fold more potent than ciprofloxacin, ofloxacin and levofloxacin against *S. pneumoniae*, with an MIC<sub>90</sub> of 0.25 mg/L. In comparison with sparfloxacin, moxifloxacin is generally two-fold more potent. However, the pharmacokinetics of moxifloxacin are more favourable than those of sparfloxacin, with four-fold higher peak concentrations in human serum.

Although fluoroquinolone resistance is not a major problem among pneumococci at this time, there are recent data suggesting that fluoroquinolone resistance may be on the increase and that this loss of susceptibility affects all clinically available fluoroquinolones. With increasing resistance in the population, it is important to evaluate the pharmacodynamics of the newer fluoroquinolones against these emerging resistant pathogens. Therefore, the purpose of this study was to compare the pharmacodynamic activity of moxifloxacin, levofloxacin and sparfloxacin against a panel of random *S. pneumoniae* clinical isolates and some isolates selected specifically for their lack of susceptibility to levofloxacin.

Using a two-compartment *in vitro* pharmacokinetic model (IVPM), oral doses of 400 mg of moxifloxacin, 500 mg of levofloxacin and 200 mg of sparfloxacin were simulated and time–kill pharmacodynamic interactions were evaluated over 36 h.

Materials and methods

*Bacterial strains and culture conditions*

The experimental strains evaluated in this study included 10 clinical isolates of *S. pneumoniae* exhibiting a wide range
of susceptibility to fluoroquinolone antibacterials, including strains that were not susceptible to levofloxacin. The susceptibilities of the 10 pneumococcal isolates to ciprofloxacin, levofloxacin, sparfloxacin and moxifloxacin are shown in Table I. Logarithmic-phase cultures were prepared by suspending 10 colonies from a 14 h culture on trypticase soy agar supplemented with 5% sheep blood (BBL Microbiology Systems, Cockeysville, MD, USA) into 6 mL of Todd–Hewitt broth (Unipath/Oxoid, Ogdensburg, NY, USA) supplemented with 0.5% yeast extract (THY). Viable bacterial counts after 10 h of incubation at 37°C in 5% CO₂ ranged from 1 × 10⁸ to 5 × 10⁸ cfu/mL.

**Antibiotic preparations and susceptibility testing**

Moxifloxacin powder was supplied by Bayer Corporation (West Haven, CT, USA). Levofloxacin powder was supplied by R. W. Johnson Pharmaceutical Research Institute (Raritan, NJ, USA). Sparfloxacin powder was supplied by Rhône-Poulenc Rorer (Collegeville, PA, USA). Antibiotic powders were dissolved in 0.2 mL of 0.1 M NaOH, diluted to final volume with distilled water and sterilized by passage through a 0.20 μm pore-size Acrodisc syringe filter membrane (Gelman Sciences, Ann Arbor, MI, USA).

Susceptibility tests with moxifloxacin, levofloxacin and sparfloxacin were performed by broth microdilution according to the procedure recommended by the National Committee for Clinical Laboratory Standards (NCCLS).8

### In vitro pharmacokinetic model

The basics of the IVPM used in this study have been described in detail.9,10 The hollow-fibre cartridges (Model # BR130; Unisyn Fibertech, San Diego, CA, USA) used in these studies consisted of 2250 cellulose acetate hollow fibres contained within a polycarbonate housing, with each fibre having 30 000 MW pores within its wall. The surface area of exchange between the fibres and the extracapillary space (peripheral compartment) was 1.5 ft². Medium containing antibiotic was pumped through the lumen of the fibres at a flow rate of 20 mL/min using Masterflex computerized peristaltic pumps (Model 7550-90; Cole-Parmer Instrument Company, Vernon Hills, IL, USA) and Easy-Load pump heads (Model 7518-00; Cole-Parmer). In addition, the bacterial culture within the peripheral compartment was continuously circulated with similar peristaltic pumps at a rate of 20 mL/min through a loop of silicone tubing attached to two ports entering and exiting the peripheral compartment. The initial volume of culture circulated through the peripheral compartment and loop of silicone tubing was 35–40 mL. When samples were required from the peripheral compartment, 0.5 mL volumes were removed through a four-way sterile stopcock (Medex, Hilliard, OH, USA) positioned within the loop of silicone tubing. The volume of THY within the central reservoir varied with each drug depending on the elimination half-life, such that the rate of dilution and elimination could be set at the minimum 0.7 mL/min allowed by the peristaltic pumps. Elimination half-lives of 12 h for moxifloxacin,5 7.5 h for levofloxacin6 and 20 h for sparfloxacin6 were simulated. The corresponding central reservoir volumes were 800 mL for studies with moxifloxacin, 450 mL for levofloxacin and 1200 mL for sparfloxacin. In drug-free control experiments, the volume of THY in the central reservoir was 500 mL and the flow rate for addition of fresh medium and elimination from the central reservoir was 2 mL/min.

**Quinolone pharmacokinetics within the IVPM**

The peak concentrations of moxifloxacin, levofloxacin and sparfloxacin achieved in human serum after single oral doses of 400 mg of moxifloxacin,5 500 mg of levofloxacin6

### Table I. Fluoroquinolone susceptibility of S. pneumoniae experimental panel

<table>
<thead>
<tr>
<th>S. pneumoniae strain number</th>
<th>moxifloxacin (mg/L)</th>
<th>levofloxacin (mg/L)</th>
<th>sparfloxacin (mg/L)</th>
<th>ciprofloxacin (mg/L)</th>
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<tr>
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<td>182</td>
<td>0.5</td>
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</tbody>
</table>

MIC (mg/L) as measured by broth microdilution assay.8
Quinolone pharmacodynamics against *Streptococcus pneumoniae*

and 200 mg of sparfloxacin were targeted in these studies. These peak concentrations were 4.5 mg/L for moxifloxacin, 6.4 mg/L for levofloxacin and 1.1 mg/L for sparfloxacin. To evaluate the pharmacokinetics of moxifloxacin, levofloxacin and sparfloxacin in the IVPM, peak concentrations of each drug were dosed into the central reservoir and samples were removed from the peripheral compartment at 0, 0.5, 1, 2, 4, 8, 12 and 24 h. Drug concentrations were measured by disc diffusion bioassay using a susceptible strain of *Escherichia coli*. The area under the concentration–time curve over 24 h (AUC₀₋₂₄) for moxifloxacin, levofloxacin and sparfloxacin were calculated using the trapezoidal rule. The AUC/MIC ratios for moxifloxacin, levofloxacin and sparfloxacin were calculated by dividing the AUC₀₋₂₄ by the MICs for specific strains of *S. pneumoniae*.12

**Pharmacodynamic experiments**

Logarithmic-phase cultures were diluted into fresh 37°C THY for a final inoculum of 1 × 10⁶–1 × 10⁷ cfu/mL, introduced into the peripheral compartment of the IVPM, and exposed to the fluoroquinolones as described above. Pharmacodynamic experiments were performed in ambient air at 37°C. At 0, 1, 2, 4, 6, 8, 24 and 36 h, samples were removed from the peripheral compartment and viable bacterial counts were measured by plating serial 10-fold dilutions of each sample into Todd–Hewitt agar (THA; BBL) and incubating plates overnight at 37°C in 5% CO₂. The lowest level of detection was 10 cfu/mL.

To prevent antibiotic carryover, samples removed from the peripheral compartment were first incubated for 15 min with 0.2 g of non-ionic polymeric adsorbent beads (Amberlite XAD-4; Sigma Chemical Co., St Louis, MO, USA). To detect the selection of mutants with decreased susceptibility to quinolones, samples removed from the peripheral compartment at 36 h were also plated onto THA containing antibiotic at a concentration 4 × MIC.

**Statistical analysis**

The LIFETEST procedure in the statistical package SAS implementing a Cox Proportional Hazards Model was used to evaluate the impact of drug, AUC/MIC ratio and peak/MIC ratio on time to eradication in these studies.

**Results**

**Characterization of *S. pneumoniae* isolates and the IVPM**

Moxifloxacin was the most potent fluoroquinolone against the 10 pneumococcal strains, with MICs ranging from 0.06 to 0.5 mg/L (Table I). Sparfloxacin was two-fold less potent than moxifloxacin against nine of the strains and equivalent to moxifloxacin against one strain. Levofloxacin was the least active fluoroquinolone, with MICs ranging from four- to 16-fold above those of moxifloxacin. Two of the strains used in this study were not susceptible to levofloxacin, with MICs of 4 mg/L.

The pharmacokinetic profiles of moxifloxacin, levofloxacin and sparfloxacin within the peripheral compartment of the IVPM are shown in Figure 1. Peak concentrations (mean ± S.E.M.) in the peripheral compartment were achieved 0.5 h after dosing into the central reservoir and were 4.5 ± 0.1 mg/L for moxifloxacin, 6.6 ± 0.2 mg/L for levofloxacin and 1.2 ± 0.1 mg/L for sparfloxacin. Calculated peak/MIC ratios ranged from 9 to 75 for moxifloxacin, from 2 to 13 for levofloxacin and from 1 to 10 for sparfloxacin (Table II). The AUC₀₋₂₄ values were 54 mg·h/L for moxifloxacin, 64 mg·h/L for levofloxacin and 20 mg·h/L for sparfloxacin. Calculated AUC/MIC ratios ranged from 108 to 900 for moxifloxacin, from 16 to 128 for levofloxacin and from 20 to 160 for sparfloxacin (Table II).

**Pharmacodynamics against *S. pneumoniae***

Pharmacodynamic data for four representative strains from the 10 clinical isolates are presented in Figure 2. Moxifloxacin was bactericidal (>3 logs of killing) against all 10 isolates of *S. pneumoniae*. With the exception of *S. pneumoniae* 167 and *S. pneumoniae* 182 (Figure 2b and d), moxifloxacin killed 99.9% of the population within 2 h after the first dose (Table II). In studies with seven of the strains, viable counts decreased at least 6 logs to below the 10 cfu/mL limit of detection (eradication) within 8 h of the first dose (Table II). In studies with two other strains, both were eradicated by moxifloxacin between 8 and 24 h after the first dose (Figure 2b and c). The only strain that moxifloxacin did not eradicate from the model was *S. pneumoniae* 182 (Figure 2d). Nevertheless, moxifloxacin still decreased viable counts of *S. pneumoniae* 182 a total of 5 logs over the 36 h experimental period. No mutants with decreased fluoroquinolone susceptibility were selected in experiments with moxifloxacin.

Levofloxacin and sparfloxacin were also bactericidal against all 10 isolates of *S. pneumoniae*. Against five strains, levofloxacin was comparable to moxifloxacin in its initial rate of killing, taking only 0.5–1 h longer to achieve a 99.9% kill. Against the remaining five strains, however, levofloxacin took anywhere from 1.5 to 5 h more time than moxifloxacin to produce a 99.9% kill. Against all strains except *S. pneumoniae* 182 (Figure 2d), sparfloxacin had the slowest rate of kill of the three fluoroquinolones, requiring up to 10 h longer than moxifloxacin to produce a 99.9% kill. Similar to moxifloxacin, sparfloxacin eventually eradicated all strains except *S. pneumoniae* 182 (Figure 2d) from the IVPM. In contrast, levofloxacin failed to eradicate the two levofloxacin-non-susceptible strains from the IVPM (Figure 2c and d). Nevertheless, despite failing to achieve eradication, levofloxacin did reduce viable counts of these non-
susceptible strains over 4 logs during the 36 h experimental period. Similar to studies with moxifloxacin, no mutants were selected in experiments with levofloxacin or sparfloxacin.

**Statistical analysis**

Statistical analysis of the factors influencing eradication in the model indicated that the three drugs were indistinguishable from each other with respect to eradication ($P = 0.14$). In contrast, AUC/MIC and peak/MIC ratios were highly significant predictors of eradication when evaluated individually in the statistical analysis ($P < 0.001$).

**Discussion**

An IVPM was used to simulate the serum pharmacokinetics of maximum oral doses of moxifloxacin, levofloxacin and sparfloxacin and to compare their pharmacodynamics against 10 clinical isolates of *S. pneumoniae*. The strains selected for this study included eight random clinical isolates and two strains selected specifically for their lack of susceptibility to levofloxacin (MIC 4 mg/L). Although fluoroquinolone resistance among *S. pneumoniae* is not currently a serious problem, remaining below 1% in large surveillance studies, there are some reports suggesting that it may be increasing. Therefore, in addition to evaluating new fluoroquinolones against random clinical isolates, it is important to evaluate them against isolates that are not susceptible to the older fluoroquinolones. Although all three fluoroquinolones bind to serum proteins (30–45% protein binding for moxifloxacin, 45% for sparfloxacin and up to 24–35% binding for levofloxacin), these pharmacodynamic studies were performed in the absence of serum proteins. While the presence of serum proteins may have altered the pharmacodynamics observed, the impact should have been similar for all three fluoroquinolones.

Moxifloxacin was rapidly bactericidal against all 10 *S. pneumoniae* in this study, eradicating most strains from the IVPM within 8 h of the first dose. These data are supported by data from Zinner and colleagues, who observed similar rates of killing and eradication of six *S. pneumoniae* isolates from a similar IVPM. Furthermore, significant killing of *S. pneumoniae* with moxifloxacin has been observed in a rabbit model of meningitis.

In comparison with moxifloxacin, levofloxacin exhibited similar rates of killing of five strains, with somewhat slower rates of killing of the other strains. The largest differences in initial rates of kill were observed against the two strains with levofloxacin MICs of 4 mg/L. Against these strains, levofloxacin required an additional 4–5 h to produce a 99.9% kill of these strains compared with moxifloxacin. However, even though the initial rates of killing with levofloxacin were slower, levofloxacin still decreased viable counts of the non-susceptible strains at least 4 logs, and by 36 h there was very little difference noted between the drugs. Sparfloxacin had the slowest rate of killing of the three fluoroquinolones, requiring up to 10 h of additional time to achieve 99.9% kills compared with moxifloxacin. The relatively faster rates of initial killing observed with moxifloxacin against some strains most likely reflects the higher peak/MIC ratios achieved with maximum doses of moxifloxacin against the *S. pneumoniae* in this study. Fluoroquinolones have been shown to exhibit a dose–response relationship in their bactericidal activity, and the enhanced potency of moxifloxacin compared with levofloxacin and more favourable pharmacokinetics compared with sparfloxacin provide better peak/MIC ratios. In addition, statistical analysis of the data from this study indicated that both the peak/MIC ratio and AUC/MIC ratio were significant predictors of eradication.

Differences in rates of killing between moxifloxacin and the other fluoroquinolones also resulted in substantially faster rates of eradication of some strains with moxifloxacin. Nevertheless, even though rates of eradication may have been slower, levofloxacin and sparfloxacin were still able to eradicate most of the *S. pneumoniae*. More...
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Figure 2. Time–kill pharmacodynamics of moxifloxacin (○) (a, c, 0.25 mg/L; b, d, 0.5 mg/L), levofloxacin (△) (a, b, 2.0 mg/L; c, d, 4.0 mg/L) and sparfloxacin (□) (a–c, 0.5 mg/L; d, 1.0 mg/L) against (a) *S. pneumoniae* 21, (b) *S. pneumoniae* 167, (c) *S. pneumoniae* 25 and (d) *S. pneumoniae* 182 (●, control). Each datum point represents the mean cfu/mL of THY from the peripheral compartment for duplicate experiments. Error bars represent standard error of the mean.
Table II. Pharmacodynamics of moxifloxacin, levofloxacin and sparfloxacin against *S. pneumoniae*

<table>
<thead>
<tr>
<th>S. pneumoniae strain no.</th>
<th>Moxifloxacin</th>
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<th>Sparfloxacin</th>
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<tr>
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<tr>
<td>182</td>
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<sup>a</sup>Ratio of peak antibiotic levels achieved within the peripheral compartment of the IVPM to the MIC of the drug for the strain of *S. pneumoniae.*

<sup>b</sup>Ratio of the AUC<sub>0–24</sub> to the MIC of the drug for the strain of *S. pneumoniae.*

<sup>c</sup>Time required to achieve a 99.9% kill of the inoculum.

<sup>d</sup>Time required to decrease viable counts below the 10 cfu/mL limit of detection.

<sup>e</sup>Eradication of the inoculum was not achieved in these experiments.
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importantly, eradication was observed despite simulated AUC/MICs of only 32–64. In previous studies, ciprofloxacin, levofloxacin and ofloxacin were shown to eradicate *S. pneumoniae* from the same IVPM consistently when AUC/MIC ratios of only 32–64 were simulated. Furthermore, Lacy and colleagues demonstrated similar eradication of *S. pneumoniae* from a similar IVPM with simulated AUC/MIC ratios as low as 29. These data suggest that the minimum AUC/MIC required for efficacy with these quinolones may be well below the minimum breakpoint of 125 suggested for ciprofloxacin against Gram-negative bacteria. This conclusion is supported by clinical data with grepafloxacin in the treatment of acute exacerbations of chronic bronchitis. Although the number of patients infected with *S. pneumoniae* was small, Forrest and colleagues reported 87.5% (7/8) bacteriological cure when AUC/MIC ratios were 92. More systematic studies evaluating the anti-pneumococcal pharmacodynamics of fluoroquinolones over a range of AUC/MIC ratios are required to determine the true minimum AUC/MIC ratio required for clinical efficacy.

In summary, the enhanced anti-pneumococcal potency of moxifloxacin compared with levofloxacin and more favourable pharmacokinetics compared with sparfloxacin provided faster rates of initial killing and faster rates of eradication of half of the *S. pneumoniae* in this study. However, despite differences in initial rates of killing and eradication, by 36 h very little difference was observed between the three fluoroquinolones in their level of killing. Further studies are required to determine whether the relatively faster rate of killing and eradication observed with moxifloxacin against some strains in this study translates into faster eradication from patients and/or more rapid clinical cure.

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**References**


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