AUC/MIC relationships to different endpoints of the antimicrobial effect: multiple-dose in vitro simulations with moxifloxacin and levofloxacin

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Most integral endpoints of antimicrobial effect, including area between the control growth and time–kill curves (ABBC), area above the curve (AAC) and area under the time–kill curve (AUBC) are determined over a dosing interval (τ), regardless of the actual effect duration. Unlike these τ-related endpoints, the intensity of antimicrobial effect (IE) considers the area between the control growth and time–kill curves from time zero to the time when bacterial counts on the regrowth curve achieve the same maximal numbers as in the absence of antibiotic, even if this time is greater than τ. Recently, important differences between ABBC, AAC and AUBC–AUC/MIC relationships were reported in single-dose simulations. The present study was designed to examine these relationships in multiple-dose simulations. A clinical isolate of Staphylococcus aureus was exposed to simulated pharmacokinetics of moxifloxacin (MIC = 0.37 mg/L) and levofloxacin (MIC = 0.6 mg/L), simulating three consecutive 24 h doses, which varied over a 32-fold range in the 24 h AUC/MIC ratio (AUC·τ/MIC: 14–444 h and 15–484 h, respectively). The cumulative effect of each treatment was expressed by IE, determined from time zero to the time after the third dose when the effect could no longer be detected, and by ABBC, AAC and AUBC calculated over a 72 h period (i.e. over three dosing intervals). With all four endpoints, systematic AUC·τ/MIC increase-induced changes in effect—an increase in IE, ABBC and AAC, or a decrease in AUBC—were observed and the log AUC·τ/MIC–response curves were fitted by an Emax model. Using IE, the effects of moxifloxacin and levofloxacin could be distinguished over a wider range of AUC·τ/MIC ratios than with ABBC and AUBC, whereas no differences between the fluoroquinolones could be seen based on the AAC–AUC·τ/MIC curves. Although ABBC and AUBC were more descriptive than AAC, these two endpoints distinguished the fluoroquinolone effects only over a relatively narrow AUC·τ/MIC range (40–100 h), which includes therapeutically achievable values for levofloxacin but not for moxifloxacin. Similar limitations of the τ-related endpoints might be critical in comparative studies with other new fluoroquinolones where therapeutic AUC·τ/MIC ratios are >100 h.

Introduction

The design and quantification of time–kill studies are often decisive in comparisons of fluoroquinolone pharmacodynamics and in the selection of optimal predictors of antimicrobial effect.1 Even related endpoints, such as the intensity of the antimicrobial effect (IE),2 the area between the control growth and time–kill curves (ABBC),3 the area above the curve (AAC) and area under the time–kill curve (AUBC5,6 sometimes abbreviated AUBKC5), produce different relationships between the antimicrobial effect of ciprofloxacin and the simulated area under the concentration–time curve (AUC).8 More recently, underestimation of the antimicrobial effect of gemifloxacin, as expressed by ABBC, AAC and AUBC determined over the simulated dosing interval (τ), was the major reason for differences in reported AUC (or AUC/MIC) relationships when compared with studies using IE, an endpoint that considers the actual duration of effect even if it is longer than τ.9

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These findings were reported in single-dose studies, where the impact of truncated areas (ABBC, AAC and AUBC) on the determination of antimicrobial effect is more dramatic than in multiple-dose simulations, where the effects of preceding doses might mask differences in the measured effect of the final dose. The present study was designed to examine whether the fundamental differences between $I_E$ and the $\tau$-related endpoints influence AUC/MIC–response relationships determined in multiple-dose simulations. Once-daily administration of moxifloxacin or levofloxacin for 3 days was simulated using a wide range of AUC/MIC ratios, and the quinolone pharmacodynamics observed with *Staphylococcus aureus* were compared in terms of the AUC/MIC relationships of $I_E$, ABBC, AAC and AUBC.

**Materials and methods**

**Antimicrobial agents, bacterial strains and susceptibility testing**

Moxifloxacin and levofloxacin powders were kindly provided by Bayer Corporation (West Haven, CT, USA) and Ortho-McNeil Pharmaceuticals (Raritan, NJ, USA), respectively. A clinical isolate of methicillin-resistant *S. aureus* (*S. aureus* 916) for which the MICs were comparable to the MIC50s of moxifloxacin (0.15 mg/L) and levofloxacin (0.7 mg/L), was selected for the study. Susceptibility testing was performed in triplicate by broth microdilution techniques at 24 h post-exposure, with the organism grown in Ca$^{2+}$- and Mg$^{2+}$-supplemented Mueller–Hinton broth at an inoculum size of 10$^6$ cfu/mL. To obtain more precise values, MICs were determined with starting concentrations of 5, 6, 7 and 8 mg/L as described earlier. The true MICs for *S. aureus* 916 determined by these multiple serial dilutions were 0.37 mg/L of moxifloxacin and 0.6 mg/L of levofloxacin.

To reveal possible changes in susceptibility during treatment, the true fluoroquinolone MICs for bacterial cultures sampled from the model were determined daily for 4 days.

**In vitro dynamic model and simulated pharmacokinetic profiles**

A dynamic model described previously was used in the study. The operation procedures, reliability of simulations of quinolone pharmacokinetic profiles and the high reproducibility of the time–kill curves provided by the model have been reported elsewhere.

A series of monoexponential profiles that simulate once-daily administration of moxifloxacin and levofloxacin for 3 days was modelled (Figure 1). The simulated half-lives (12.1 h for moxifloxacin and 6.8 h for levofloxacin) represented weighted means of values reported in humans (9.1–13.4 h$^{13,14}$ and 6.0–7.4 h$^{15,19}$ respectively). The rates of fresh nutrient medium influxed into the 60 mL central compartment and the antibiotic- and bacteria-containing medium effluxed from this compartment were 3.5 mL/h and 6.1 mL/h, respectively. Daily doses of the quinolones were designed to correspond to comparable mean values of the AUC$_\tau$/MIC ratio (average of values reached after the first, second and third doses): 14–444 h with moxifloxacin and 15–484 h with levofloxacin, where $\tau$ is a 24 h dosing interval.

**Determination of time–kill curves and the antimicrobial effect**

In each experiment multiple sampling of the bacteria-containing medium from the central compartment was performed throughout the observation period. The duration of the experiments, in each case, was defined as the time until the numbers of antibiotic-exposed bacteria, after the third dose, reached the maximum observed in the absence of antibiotic ($\geq 10^9$ cfu/mL). The lower limit of accurate detection was $2 \times 10^2$ cfu/mL.

Based on time–kill data, ABBC, AAC and AUBC were determined for each dosing interval, i.e. from time zero to 24 h, from 24 to 48 h and from 48 to 72 h. The cumulative antimicrobial effect (0–72 h) was defined as the sum of these partial ABBC, AAC or AUBC (ABBC$_\tau$, AAC$_\tau$ and AUBC$_\tau$).

![Figure 1. In vitro simulated pharmacokinetic profiles of moxifloxacin and levofloxacin. The arrows reflect quinolone multiple dosing. The number at each plot indicates the simulated AUC$_\tau$/MIC ratio (in hours).](https://academic.oup.com/jac/article-abstract/50/4/533/761649/534)
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respectively). Unlike these τ-related endpoints of antimicrobial effect, was determined from time zero to the time when the effect could no longer be detected, i.e. the time after the third quinolone dose at which the number of antibiotic-exposed bacteria reached 10⁶ cfu/mL. The upper limit of bacterial numbers, i.e. the cut-off level on the regrowth and control growth curves used to determine Iₕ and ABBC, or on the regrowth curve only for AAC and AUBC determination, was 10⁹ cfu/mL. If counts were lower, they were extrapolated to the cut-off level using a logistic function. The computation of Iₕ, ABBC, AAC and AUBC at two simulated AUC/τ/MIC ratios of moxifloxacin is depicted graphically in Figure 2.

Relationships of the antimicrobial effect to the AUC/τ/MIC ratio

For both quinolones, the endpoints of antimicrobial effect were fitted to the log AUC/τ/MIC ratios using an Eₘₐₓ model:

\[ Y = E_{\text{max}} + (E_{\text{min}} - E_{\text{max}}) \frac{1}{1 + \exp \left(\frac{x - x_{50}}{d} \right)} \] (1)

where \( x \) is the log AUC/τ/MIC ratio, \( Y \) is Iₕ, ABBC, AAC or AUBC, \( E_{\text{max}} \) and \( E_{\text{min}} \) are the maximal and minimal values of the antimicrobial effect, \( x_{50} \) is \( x \) corresponding to \( E_{\text{max}}/2 \) and \( d \) is the parameter reflecting width.

Quasi-linear portions of the Iₕ-log AUC/τ/MIC ratio curves, i.e. Iₕs at AUC/τ/MIC ratios > 100 h, were fitted by the linear equation

\[ I_{\text{h}} = a + b \log (\text{AUC}/\tau/\text{MIC}) \] (2)

where \( a \) and \( b \) are intercept and slope parameters, respectively.

Results

Time–kill curves

The time courses of viable count, which reflect killing and regrowth of S. aureus exposed to moxifloxacin and levofloxacin administered once daily, are shown in Figure 3. At lower AUC/τ/MIC ratios, from 14 to 28 h with moxifloxacin and 15 to 61 h with levofloxacin, regrowth occurred during each dosing interval following either a negligible (at the lowest AUC/τ/MIC ratio of moxifloxacin) or a considerable reduction in bacterial numbers (at a moxifloxacin AUC/τ/MIC ratio of 28 h and levofloxacin AUC/τ/MIC ratios of 15–61 h). At an AUC/τ/MIC ratio of 56 h, moxifloxacin gave more pronounced reductions in bacterial count after the first and second doses, with regrowth occurring during the third dosing interval. No regrowth occurred during the third dosing intervals when higher AUC/τ/MIC ratios were used for moxifloxacin (111–444 h) and levofloxacin (121–484 h). However, at all these AUC/τ/MIC ratios except for the highest for moxifloxacin (444 h), bacterial regrowth was observed on the fourth day.

As seen in Figure 3, at the lowest AUC/τ/MIC ratio (14 h with moxifloxacin and 15 h with levofloxacin) similar reductions in bacterial numbers were observed during the first, second and third dosing intervals. At higher AUC/τ/MIC ratios with both drugs (moxifloxacin at 28 h and levofloxacin at 31 and 61 h), the reduction in bacterial numbers after the third dose and, to a lesser extent after the second dose, was more pronounced than after the first dose. A slightly decreased antimicrobial effect during the third dosing interval was observed with moxifloxacin at an AUC/τ/MIC ratio of 56 h, although the reductions in bacterial numbers observed during the first and second dosing intervals were similar. No degradation of effect was observed during the second and third dosing intervals with AUC/τ/MIC ratios ≥ 111 h (moxifloxacin) and 121 h (levofloxacin). These features of the time–kill curves are illustrated by plotting partial ABBC against the time after the
beginning of the 3 day fluoroquinolone treatment (Figure 4). As seen in the figure, systematically decreasing ABBC occurred with AUC/\text{MIC} ratios of 28 h for moxifloxacin and 31 and 61 h for levofloxacin. At lower AUC/\text{MIC} ratios, the ABBC plots were parallel to the time axis, whereas at higher AUC/\text{MIC} ratios, a slight increase in the ABBC was seen during the third dosing interval.

Repeated susceptibility testing showed an increased true MIC of moxifloxacin (>0.75 mg/L) on the fourth day after treatment with an AUC/\text{MIC} ratio of 28 h, without any changes at other simulated AUC/\text{MIC} ratios. Unlike moxifloxacin, slightly increased MICs of levofloxacin (>0.75 mg/L) were identified at AUC/\text{MIC} ratios of 31, 61 and 121 h.

**Relationships of the antimicrobial effect to AUC/\text{MIC} ratio**

The AUC/\text{MIC} ratio relationships of the cumulative antimicrobial effect, expressed by \(I_E\), ABBC, AAC and AUBC and fitted by equation (1), are shown in Figure 5 and the respective best-fit estimates of \(E_{\text{max}}\), \(E_{\text{min}}\), \(x_{50}\) and \(dx\) are presented in Table 1. As seen in Figure 5, the \(I_E\)-log AUC/\text{MIC} ratio curves display less sigmoidicity than the ABBC-, AAC- and AUBC-log AUC/\text{MIC} ratio curves. Moreover, the same 32-fold range in the simulated AUC/\text{MIC} ratios is associated with larger ranges of \(I_E\) from 0 to \(-600\) (log cfu/mL) × h for moxifloxacin and \(-550\) (log cfu/mL) × h for levofloxacin, than of ABBC \([0–425\) (log cfu/mL) × h], AAC \([-160–210\) to 200–220 (log cfu/mL) × h] or AUBC \([600–640\) to 220–230 (log cfu/mL) × h]. Based on the \(I_E\)-log AUC/\text{MIC} ratio relationships, the different effects of moxifloxacin and

![Figure 4. ABBC at different AUC/\text{MIC} ratios simulated with moxifloxacin and levofloxacin.](https://academic.oup.com/jac/article-abstract/50/4/533/761649)
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levofloxacin are seen distinctly over most of the range of simulated AUC/\(\text{MIC}\) ratios (40–400 h). However, with the \(\tau\)-related endpoints, the AUC/\(\text{MIC}\) ratio relationships display differences only over a relatively narrow range (40–100 h; ABBC and AUBC) or they do not distinguish between the quinolones at all (AAC).

Discussion

Like single-dose experiments,\(^{10}\) time–kill curves of \(S.\) aureus exposed to multiple doses of moxifloxacin and levofloxacin differed more in times to regrowth than in the rate and extent of bacterial killing (Figure 3). Also, with both fluoroquinolones, these regrowth times correlated with simulated AUC/\(\text{MIC}\) ratios: the greater the AUC/\(\text{MIC}\) ratio, the greater the time to regrowth after the final dose. Moreover, at comparable AUC/\(\text{MIC}\) ratios (56 and 61 h, 111 and 121 h or 222 and 242 h for moxifloxacin and levofloxacin, respectively), the times to regrowth observed after moxifloxacin treatment were all longer than those observed with levofloxacin. Systematically decreasing ABBC\(_\tau\)s (Figure 4) were observed at a moxifloxacin AUC/\(\text{MIC}\) ratio of 28 h and levofloxacin AUC/\(\text{MIC}\) ratios of 31 and 61 h, i.e. precisely at those AUC/\(\text{MIC}\) ratios reported to be associated with increased MIC or selection of resistant bacteria. The levofloxacin AUC/\(\text{MIC}\) ratio of 121 h also selected for organisms for which the MIC was slightly increased. This suggests that the AUC/\(\text{MIC}\) ratio-induced differences in the ABBC\(_\tau\) time course may correlate with the selection of resistant staphylococci after fluoroquinolone exposure.

Similar differences in the AUC/\(\text{MIC}\)–response curves were seen when using different endpoints for antimicrobial effect (Figure 5) in single-dose simulations with gemifloxacin.\(^{9}\) The largest differences in effect areas, corresponding to the simulated AUC/\(\text{MIC}\) ratios, were seen when using \(I_E\) as the endpoint. This appears to be the most descriptive outcome measure. Using \(I_E\), the effects of moxifloxacin and levofloxacin could be distinguished over a wider range of AUC/\(\text{MIC}\) ratio than with ABBC and AUBC endpoints, whereas no differences in effect could be seen between the two agents with the AAC–AUC/\(\text{MIC}\) ratio curves. Although ABBC and AUBC endpoints were more descriptive than AAC, the former two endpoints could distinguish the fluoroquinolone effects only over a narrow AUC/\(\text{MIC}\) ratio range (40–100 h). Given the difference in therapeutic AUC/\(\text{MIC}\) ratios seen with 500 mg levofloxacin and 400 mg moxifloxacin against staphylococci (AUC/\(\text{MIC}\)\(_{50}\) ratios of 65 and 205 h\(^{20}\)), these values fall in the therapeutic range for levofloxacin but not for moxifloxacin. This might also be a limitation when making comparisons between the other new fluoroquinolones that have larger therapeutic AUC/\(\text{MIC}\)\(_{50}\) ratios, such as 130 h for grepafloxacin, 140 h for gatifloxacin, 190 h for trovafloxacin and 310 h for gemifloxacin.\(^{20}\) The fact that these values all fall

![Figure 5. AUC/\(\text{MIC}\) ratio-dependent endpoints of the antimicrobial effect of moxifloxacin (bold lines) and levofloxacin (thin lines) fitted by equation (1).](https://academic.oup.com/jac/article-abstract/50/4/533/761649/537)
in the plateau portion of the ABBC− and AUBC−AUC$/\text{MIC}$ curves (Figure 5) precludes accurate fluoroquinolone comparisons and clinically relevant predictions. As shown previously,\textsuperscript{8} this plateau results from the underestimation of the true antimicrobial effect with the use of $\tau$-related endpoints when the effect actually persists longer than $\tau$, in this case, $3 \times \tau$. ABBC, AAC and AUBC all consider the truncated areas after the third dose and ignore substantial portions of the actual effect (Figure 2). However, as mentioned above, the $\tau$-related endpoints may be useful to compare the effects provided by each subsequent dose of quinolone. Although the systematic decrease in antimicrobial effect over the course of treatment with moxifloxacin (AUC$/\text{MIC}$ ratio 28 h) and levofloxacin (AUC$/\text{MIC}$ ratios of 31 and 61 h) was demonstrated using ABBC, a similar conclusion might be drawn using AUBC.

As reported previously,\textsuperscript{1} $I_E$ analysis provides predictions of fluoroquinolone effects that may be relevant clinically. Recently, in single-dose simulations with moxifloxacin and levofloxacin,\textsuperscript{10} AUC$/\text{MIC}$ ratio values of 80 and 130 h, respectively, were predicted as equivalent to the reported ciprofloxacin AUC$/\text{MIC}$ ratio breakpoint of 125 h.\textsuperscript{21} To verify these predictions, a similar analysis was performed in this multiple-dose study. Although the $E_{\text{max}}$ model was used to fit the $I_E$ versus log AUC$/\text{MIC}$ ratio over the entire AUC$/\text{MIC}$ ratio range, at AUC$/\text{MIC}$ ratios $>50$ h, the $I_E$−log AUC$/\text{MIC}$ ratio data may be approximated by a linear function (Figure 6). A levofloxacin AUC$/\text{MIC}$ ratio of 120 h, which corresponds to an AUC/MIC ratio of 130 h, provides the same $I_E$ as a moxifloxacin AUC$/\text{MIC}$ ratio of 60 h, which in turn, corresponds to an AUC/MIC ratio of 80 h. Thus, the AUC/MIC ratio breakpoints predicted in single and multiple-dose studies using $I_E$−log AUC$/\text{MIC}$ ratio or $I_E$−log AUC$/\text{MIC}$ ratio relationships are very similar.

Overall, this study demonstrates the rapid and sustained bactericidal action of moxifloxacin and levofloxacin at clinically relevant concentrations, with a greater $I_E$ (intensity of antimicrobial effect) for moxifloxacin when compared with levofloxacin.

**Acknowledgements**

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**References**


### Table 1. Best-fit estimates of the parameters of equation (1) $\pm$ S.E.M. $(P = 0.05)$

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<th>Endpoint</th>
<th>Fluoroquinolone</th>
<th>$E_{\text{max}}$</th>
<th>$E_{\text{min}}$</th>
<th>$x_{50}$</th>
<th>$dx$</th>
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<td>$I_E$</td>
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<td>591 $\pm$ 32.3</td>
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<td>1.66 $\pm$ 0.04</td>
<td>0.18 $\pm$ 0.03</td>
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<td>levofloxacin</td>
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<td>0.28 $\pm$ 0.03</td>
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<td>1.53 $\pm$ 0.01</td>
<td>0.11 $\pm$ 0.01</td>
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<td>levofloxacin</td>
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<td>1.62 $\pm$ 0.02</td>
<td>0.21 $\pm$ 0.02</td>
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**Figure 6.** Approximation of the $I_E$−log AUC$/\text{MIC}$ ratio data by equation (2) and prediction of the equivalent AUC$/\text{MIC}$ ratio breakpoints.
Endpoints of fluoroquinolone antimicrobial effect


