Introduction

Moxifloxacin (Bayer name: Bay 12-8039) is an 8-methoxy-quinolone which, when compared with ciprofloxacin, has improved in-vitro activity against Gram-positive bacteria, atypical respiratory pathogens and anaerobes. The MIC values for methicillin- and ciprofloxacin-resistant Staphylococcus aureus (MSSA) are 0.03 mg/L, but for methicillin- and ciprofloxacin-resistant S. aureus (MRSA) are 4 mg/L. MIC values for Group A streptococci are 0.25 mg/L. Moxifloxacin is known to be rapidly bactericidal against S. aureus, but less so against Group A streptococci, when constant concentrations of 0.5× and 10× MIC are used. In addition, at concentrations of 1×, 4× and 10× MIC the post-antibiotic effect against S. aureus is 0.9–3.3 h and for Group A streptococci 0.3–3.3 h.

Previous work using in-vitro models has concentrated on studying the antibacterial effect of moxifloxacin on respiratory tract pathogens such as Streptococcus pneumoniae or H. influenzae and M. catarrhalis. This study focused on pathogens likely to be associated with skin and soft tissue infection, i.e. S. aureus or β-haemolytic streptococci.

The serum concentrations associated with the oral administration of 400 mg moxifloxacin every 24 h over 48 h in man were simulated in an in-vitro dilutional, continuous bacterial culture model of infection. The initial inoculum was 5 × 10⁷-5 × 10⁸ cfu/mL and all strains were tested on at least three occasions. Two strains of Staphylococcus aureus (one methicillin susceptible, the other resistant) with moxifloxacin MICs 0.14 mg/L and 0.06 mg/L and two strains of β-haemolytic streptococci, Lancefield Group A, MIC 0.16 mg/L and Group G, MIC 0.4 mg/L were used. In addition, two laboratory-generated mutants with raised moxifloxacin MICs were also employed: methicillin-sensitive S. aureus (MSSA) MIC 1.0 mg/L and Group A streptococcus MIC 1.8 mg/L. The antibacterial effect of moxifloxacin was judged by changes in viable count over time, and the area under the bacterial-kill curve (AUBKC) after 24 and 48 h. For S. aureus MIC 0.14 mg/L the AUBKC of the MSSA strain was 77.8 ± 4.6 and AUBKC of the MRSA strain, 92.0 ± 6.9. For its mutant, moxifloxacin MIC 1.0 mg/L, the AUBKC was 116.1 ± 15.6 and AUBKC of the MRSA strain, 211.9 ± 23.1, indicating decreased killing. AUBKC of the MSSA and AUBKC of the MRSA strain, 110.7 ± 10.3 and 130.9 ± 21.3, respectively, were noted for the MSSA strain. The Group A streptococcus, MIC 0.16 mg/L, had an AUBKC of 91.4 ± 19.4 and AUBKC of 157.0 ± 70.9. The mutant, MIC 1.8 mg/L, had an AUBKC of 127.0 ± 1.9 and AUBKC of 205.1 ± 6.4. Despite a lower MIC (0.4 mg/L) the single strain of Group G streptococcus tested was killed poorly, AUBKC of 139.9 ± 3.6 and AUBKC of 252.0 ± 18.6. The pharmacodynamic parameters AUC/MIC, T > MIC, (AUC > MIC)/MIC (AUC = area under the curve, T = time) and WAUC (weighted area under the curve) were related to the MRSA strain. T > MIC was poorly related to AUBKC (r = 0.36) while AUC/MIC, (AUC > MIC)/MIC and WAUC were strongly related to AUBKC (r = 0.75–0.79) and AUBKC (r = 0.78–0.84). The maximum antibacterial effect was achieved with an AUC/MIC ratio of 150–200. AUC-related pharmacodynamic parameters predicted antibacterial effect better than T > MIC.
streptococci. The antibacterial effect of moxifloxacin was assessed by simulating changing concentrations of antibiotic in an in-vitro pharmacodynamic model of infection. As in previous studies the serum concentrations of moxifloxacin associated with doses of 400 mg administered once a day were modelled, as this is the dose of moxifloxacin used most commonly in human clinical trials.

Materials and methods

In-vitro model

An in-vitro model (New Brunswick Bioflo 1000; Hatfield, UK) was used to simulate oral administration of moxifloxacin 400 mg every 24 h over 48 h; i.e. two simulated doses. The apparatus consists of a single central culture chamber connected via aluminium and silicone tubing first to a dosing chamber (which is in turn connected to a reservoir containing broth) and secondly to a vessel collecting outflow broth from the central chamber. The dosing chamber and central culture chamber were diluted with broth from the reservoir using a peristaltic pump (Ismatec, Switzerland) and stored at –70ºC.

Moxifloxacin (1332 μg/mL) and the model run for 18 h to allow equilibrium to be attained at a density of about 5 \times 10^5 cfu/mL. Moxifloxacin (1332 μg/mL of a 1 g/L solution) was then added to the dosing chamber at time 0, and at 24 h. Samples were taken from the central chamber via a port throughout the 48 h period (at times 0, 1, 2, 3, 4, 5, 6, 7, 10, 12, 22, 24, 25, 26, 27, 28, 29, 30, 31, 34, 36, 46 and 48 h) for assessment of viable bacterial count. The bacteria were counted without dilution and after 1/100 dilution using a spiral plater (Don Whitley Spiral Systems, Shipley, UK). The minimum detection level was 2 × 10^2 cfu/mL. In addition, aliquots were taken at the same time intervals and stored at –70ºC for measurement of moxifloxacin concentration. Samples were assayed by bioassay using E. coli NCTC 10418 as indicator organism. All standards and samples were prepared and diluted as necessary in the same concentration of BHI or ISB as that used in the model simulations. The limit of detection was 0.03 mg/L. All pharmacokinetic simulations and bacterial killing experiments were performed at least three times.

Pharmacokinetics and bacterial killing curves

The in-vitro activity of changing concentrations of moxifloxacin against the six strains was tested in the model described above. Concentrations were chosen to simulate serum concentrations in man after a 400 mg oral dose every 24 h (to give a target peak serum concentration of 2.5 mg/L at 2 h post dose and a 24 h post-dose concentration of 0.4 mg/L). The area under the curve (AUC) was 24.4 mg/L.

For all the experiments, 100 μL of an overnight broth suspension of the test organism was inoculated into the central culture chamber via an entry port (initial inoculum of about 10^5 cfu/mL) and the model run for 18 h to allow equilibrium to be attained at a density of about 5 \times 10^5-5 \times 10^6 cfu/mL. Moxifloxacin (1332 μg/mL of a 1 g/L solution) was then added to the dosing chamber at time 0, and at 24 h. Samples were taken from the central chamber via a port throughout the 48 h period (at times 0, 1, 2, 3, 4, 5, 6, 7, 10, 12, 22, 24, 25, 26, 27, 28, 29, 30, 31, 34, 36, 46 and 48 h) for assessment of viable bacterial count. The bacteria were counted without dilution and after 1/100 dilution using a spiral plater (Don Whitley Spiral Systems, Shipley, UK). The minimum detection level was 2 × 10^2 cfu/mL. In addition, aliquots were taken at the same time intervals and stored at –70ºC for measurement of moxifloxacin concentration. Samples were assayed by bioassay using E. coli NCTC 10418 as indicator organism. All standards and samples were prepared and diluted as necessary in the same concentration of BHI or ISB as that used in the model simulations. The limit of detection was 0.03 mg/L. All pharmacokinetic simulations and bacterial killing experiments were performed at least three times.

Pharmacokinetics, pharmacodynamic measurement of antibacterial effects and statistical analysis

Antibiotic

Moxifloxacin was obtained from Bayer A G, Wuppertal, Germany. Stock solutions were prepared according to The British Society of Antimicrobial Chemotherapy (BSAC) guidelines and stored at –70ºC.

MICs

MICs were determined by the BSAC-defined standard broth dilution method modified to use moxifloxacin concentrations that decreased by 0.2 mg/L or 0.02 mg/L steps rather than doubling dilutions.

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Moxifloxacin pharmacodynamics

cfu/mL h) was calculated, after the inoculum was standardized, by the log-linear trapezoidal rule for the time periods 0–24 h (A UbKc24) and 0–48 h (A UbKc48). For pharmacodynamic analysis the AUC/MIC ratio, the percentage of time the simulated concentration exceeded the MIC (T > MIC) (T = time), the AUC over MIC, MIC ratio ((AUC > MIC)/MIC) and weighted AUC: WAUC = (AUC/MIC × T > MIC/100) were also calculated.12

A U C / M I C , T > M I C , ( A U C > M I C ) / M I C and WAUC were related to A UbKc24 and A UbKc48 using an inhibitory sigmoid E max model (WinNonlin, Microsoft Corp., USA). Goodness of fit was assessed by r and inspection of the plot of the residuals.

Results

Pharmacokinetic data

Moxifloxacin was detected at a concentration of 2.3 ± 0.5 mg/L at 2 h and 0.5 ± 0.1 mg/L at 24 h. These levels correlated well with those expected.

Bacterial killing curves

The killing curves for S. aureus after exposure to moxifloxacin are shown in Figures 1 and 2. For the two strains with moxifloxacin MIC ≤ 0.14 mg/L there was a marked reduction in viable count (6 log) at 36 h with no regrowth occurring up to 48 h. In contrast, killing of the laboratory-generated mutant (MIC 1.0 mg/L) was reduced (2 log reduction in count), with marked regrowth between 36 and 48 h. The A UbKc values were less for the more susceptible strains when compared with the mutant strains, especially A UbKc48. Results for the Group A streptococci tested showed that the more susceptible strain (MIC 0.16 mg/L) was killed more rapidly (3.5 log10) with no regrowth. The mutant strain (MIC 1.8 mg/L) showed marked regrowth (2 log) and was poorly killed between 0 and 24 h (Figure 3). For these simulations a significant between-experiment variability was exhibited (Figure 3 and Table). Killing of Group G streptococci (MIC 0.4 mg/L) was poor, with only a 2 log reduction in count over 48 h (Figure 4 and Table).

Pharmacodynamics of the antibacterial effect

Curve fitting using an inhibitory E max sigmoid model indicated that T > MIC was poorly related to A UbKc24 and A UbKc48 (r <0.37). A UC/MIC, WAUC and (AUC > MIC)/MIC were strongly related to A UbKc24 (r = 0.75–0.79) and A UbKc48 (r = 0.78–0.84). The plot of AUC/MIC versus A UbKc24 is shown in Figure 5. Inspect...
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Discussion

Previous data have indicated that moxifloxacin may be less bactericidal for Group A streptococci than for S. aureus even if allowance is made for differing MIC values. Our data support this view—killing of a Group A streptococcus (MIC 0.16 mg/L) was remarkably less than that of a strain of S. aureus with an MIC of 0.14 mg/L. At higher MICs, no differences were observed, probably because moxifloxacin was not especially bactericidal for either species. While other data from in-vitro models are mostly lacking for S. aureus or β-haemolytic streptococci, it is clear that moxifloxacin at in-vitro doses of 400 mg/24 h is rapidly bactericidal for S. pneumoniae with MICs ≤ 0.5 mg/L. In

Figure 4. Bactericidal effect of moxifloxacin at a simulated dose of 400 mg every 24 h on β-haemolytic streptococcus (Group G) strain 14230 (MIC 0.4 mg/L). Bacterial counts are mean ± s.d.

![Figure 4](image_url)

Figure 5. M oxifloxacin antibacterial activity against S. aureus and β-haemolytic streptococci: relationship between A UBKC and AUC/MIC using a sigmoid inhibitory E\textsubscript{max} model.

![Figure 5](image_url)
Moxifloxacin pharmacodynamics

these experiments β-haemolytic streptococci, Lancefield Group A MIC 0.16 mg/L or Group G MIC 0.4 mg/L, were not eradicated after two exposures to moxifloxacin. In contrast, using the same model, we were able to demonstrate significant bactericidal action of moxifloxacin at up to 36 h, using S. pneumoniae strains with MICs ≤ 0.5 mg/L. Using simulations of 100 mg or 200 mg single doses, it has been shown previously that S. aureus can be eradicated from an in-vitro model within 12 h of dosing. Our data confirm that there is significant bactericidal activity at 36-48 h for S. aureus strains with MICs ≤ 0.14 mg/L.

A nalinal, in which E. coli, Klebsiella pneumoniae, S. aureus and S. pneumoniae are used in neutropenic mouse models of lung or thigh infection, have indicated that the dose required to produce a net bacteriostatic effect over 24 h is unchanged, whether moxifloxacin is given every 3, 6 or 12 h. In mice, moxifloxacin has a serum half-life of <1 h compared with 12-15 h in man. This probably indicates that once-daily dosing will be as effective as twice daily or more frequent doses. Drug AUC correlated with antibacterial efficacy more strongly than did Cmax or T > MIC. Furthermore, in an experimental animal model of S. pneumoniae meningitis, the drug AUC in cerebrospinal fluid correlated with the change in viable count. Previous studies by this group, using the model system described here, have shown that the antibacterial effect of moxifloxacin correlated best with AUC/MIC for H. influenzae and M. catarrhalis. These data also show that AUC parameters correlate best with antimicrobial effect, whereas relative to other parameters as has been postulated recently. This is perhaps not surprising, as T > MIC is an integral part of WAUC. A study of this study is not of a dose fractionation or escalation design, it was not possible to define the relative roles of Cmax/MIC ratio in comparison with AUC/MIC, despite recent interest in this area.

In conclusion, moxifloxacin has marked bactericidal action against S. aureus with MICs ≤ 0.14 mg/L but may be less bactericidal against β-haemolytic streptococci; AUC-based pharmacodynamic factors predict antibacterial effect better than does T > MIC.

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References


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