Comparative In Vitro and In Vivo Activity of the C-8 Methoxy Quinolone Moxifloxacin and the C-8 Chlorine Quinolone BAY y 3118

Axel Dalhoff
Bayer AG, Pharma Research Center Wuppertal, Germany

The C-8 methoxy quinolone moxifloxacin is highly bactericidal against wild-type and first-step gyrase- and topoisomerase IV-resistant mutants. This finding led to the hypothesis that the C-8 methoxy group may lower the propensity for resistance development compared with quinolones possessing different substituents at the C-8 position. Therefore, resistance development of the C-8 methoxy quinolone moxifloxacin was compared with that of its structural analogue BAY y 3118 (chlorine moiety at the C-8 position), with Staphylococcus aureus used as the test organism. The spontaneous emergence of resistance was quantified by counting the number of colonies growing on drug-free medium compared with moxifloxacin- or BAY y 3118-containing media. The multistep emergence of quinolone resistance was encountered by growing S. aureus over 8 passages in drug-containing medium. Human serum concentrations were simulated in an in vitro model over 84 h (dosing every 24 h), and total and resistant S. aureus were quantified. Spontaneous mutation frequencies of $6 \times 10^{-11}$ for moxifloxacin and $4 \times 10^{-7}$ for BAY y 3118 were observed. Multistep resistance to moxifloxacin developed slowly (2-fold rise) but rapidly against BAY y 3118 (>16-fold rise). No resistance against moxifloxacin developed in this model, whereas resistance to BAY y 3118 began to develop after 4 h. Thus, as the C-8 moiety was the only difference, the 8-methoxy group on moxifloxacin appeared to significantly lower the propensity for quinolone resistance development.

The development of resistance to specific antibiotics continues to be a growing problem. In an effort to respond to this problem, the past decade has seen the search for and development of many new quinolones. Monofluorinated quinolones, such as ciprofloxacin, are predominantly active against gram-negative pathogens; dihalogenated and trihalogenated quinolones, on the other hand, have greatly enhanced antibacterial activity and pharmacokinetics in humans. However, the development of many of these promising dihalogenated and trihalogenated agents, including BAY y 3118, was discontinued because of adverse events, including phototoxicity and other unexpected adverse reactions [1].

New quinolones carrying a methoxy substituent at the C-8 position are photostable and do not cause phototoxicity [2]. Moxifloxacin is an 8-methoxy quinolone that is characterized by a safety profile comparable to that of older monofluorinated quinolones [3].

In addition to the thorough investigation of the safety profile, antibacterial spectrum, and pharmacodynamics of new antimicrobial drugs, it is imperative to evaluate their ability to select for mutant strains. The past few years have witnessed a dramatic increase worldwide in the incidence of resistance among both nosocomial and community-acquired pathogens [4–6]. The selection of
resistant bacteria should be minimized by the appropriate use of antibacterials and by the selection of drugs with maximal activity and optimal pharmacodynamics. Furthermore, the selection of resistant bacteria could be minimized by the development of derivatives with a lower propensity for resistance development.

It has been demonstrated recently that moxifloxacin shows increased bacteriostatic and bactericidal activities against gram-positive bacteria when compared with previous quinolones, including borderline susceptible strains and first-step mutants [7]. Here we tested the hypothesis that the C-8 methoxy substitution selects mutants much less frequently than does a halogen substitution by comparing the propensity for resistance development between moxifloxacin and BAY y 3118, which differ structurally only in the group found at the C-8 position of the molecule (figure 1).

**MATERIALS AND METHODS**

**Bacterial strains.** The following Staphylococcus aureus strains were selected from a culture collection (Department for Antibacterial Research, Bayer AG, Wuppertal, Germany) because of their identical susceptibilities to both moxifloxacin and BAY y 3118: S. aureus 133 and S. aureus 12241 (quinolone and \( \beta \)-lactam susceptible), S. aureus 25927 (methicillin-resistant S. aureus and ciprofloxacin susceptible), and S. aureus 25894 (methicillin-resistant S. aureus and ciprofloxacin resistant). S. aureus 133 was chosen as an infecting organism for the in vivo experiments because the other strains were not viable in the pouch exudate. To develop fluoroquinolone-resistant S. aureus 133, bacteria were cultivated as described below (multistep emergence of resistance). The MICs of moxifloxacin against S. aureus 133 rose to 1 mg/L after 23 transfers.

**Spontaneous emergence of resistance.** Fluoroquinolone-resistant variants were elicited by spreading an inoculum of \( 10^9 - 10^{11} \) cfu/mL on Isosensitest agar (Oxoid) incorporating the 2 quinolones at 4 times the individual MICs of the test organism S. aureus 133; the MICs for both quinolones were 0.06 \( \mu \)g/mL. After overnight incubation in ambient air at 37°C, the colonies growing on the plates were counted. The frequency of appearance of resistant variants was calculated as the ratio of resistant variants arising after overnight incubation to the number of cfu originally inoculated.

**Multistep emergence of resistance.** To characterize the emergence of multistep fluoroquinolone resistance, S. aureus 133 was grown overnight in brain-heart infusion broth (Oxoid) containing 2-fold dilutions of the quinolones ranging from 128 to 0.01 mg/L (analogous to MIC determinations). From the test tube containing the highest drug concentration permitting visible growth (i.e., 0.5 times the MIC), aliquots were used after a 1:20 dilution to inoculate a second set of serial drug dilutions. After an overnight incubation, bacteria were transferred sequentially for a period of 5 days. After cessation of the serial transfers, bacteria with the highest MICs were subcultured daily on quinolone-free agar to assess the stability of quinolone resistance.

**Emergence of resistance during exposure to fluctuating concentrations in vitro.** To simulate human serum concentrations after oral administration, a slightly modified 1-compartment model according to Grasso et al. [8] was used. This model consists of a central compartment into which the antibiotics to be studied are pumped via programmable pumps until the maximum serum concentrations in vitro. To simulate human serum concentrations after oral administration, a slightly modified 1-compartment model according to Grasso et al. [8] was used. This model consists of a central compartment into which the antibiotics to be studied are pumped via programmable pumps until the maximum serum concentration to be simulated is reached. Thereafter, antibiotic-free medium is pumped into the central compartment and is continuously eliminated in parallel to mimic half-life (\( t_{1/2} \)) values. Control growth in the absence of antibiotics was studied in the same model.

The following parameters were simulated after oral doses of moxifloxacin, where \( C_{\text{max}} \) is the peak concentration and \( t_{\text{max}} \) is the maximum time: 100 mg – \( C_{\text{max}} = 0.5 \) mg/L; 200 mg – \( C_{\text{max}} = 1 \) mg/L; 400 mg – \( C_{\text{max}} = 2 \) mg/L; 600 mg – \( C_{\text{max}} = 3 \) mg/L; \( t_{\text{max}} = 2.5 \) h; \( t_{1/2} = 11.5 \) h for once-daily administration. For BAY y 3118, the values were as follows: 200 mg – \( C_{\text{max}} = 0.84 \) mg/L; \( t_{\text{max}} = 1.5 \) h; \( t_{1/2} = 11 \) h.

**In vivo emergence of resistance.** The rat pouch model was used to monitor the growth of the organisms and the development of resistance within individual animals over longer times [9–11]. Female Wistar rats weighing 120–160 g (Winkelmann) were used. Inocula of S. aureus 133 wild type or its first-step and multistep mutant strain, respectively, were used to produce initial viable counts of \( \sim 10^8 \) cfu/mL exudate. Treatment was started

![Figure 1. Fluoroquinolone structures, with the C-8 position indicated (arrow).](image-url)
either 1 h after infection or delayed for 24 h after infection. An oral dose of moxifloxacin or BAY y 3118 (100 mg/kg/day) was administered to groups of 10 rats, but because of the rapid reduction in viable counts produced by this dose, a lower dose (50 mg/kg/day) was administered, starting 24 h after infection, to additional groups of 10 animals. The duration of treatment was 7 days when treatment started 1 h after infection, or 6 days when treatment was delayed for 24 h.

In summary, the following regimens were studied against S. aureus by use of wild-type, first-step, and multistep mutants: moxifloxacin, 100 mg/kg, started 1 h after infection; moxifloxacin, 100 mg/kg, started 24 h after infection; and moxifloxacin, 50 mg/kg, started 24 h after infection.

Exudate samples were withdrawn daily immediately before administration of the drug and plated onto drug-free agar plates (total cfu) or onto plates containing moxifloxacin (2 times or 4 times the MIC) to test for the emergence of resistance. In addition, the concentrations of moxifloxacin or BAY y 3118 in the pouch exudates were determined with a conventional cup-plate agar diffusion method with Bacillus subtilis as the indicator organism, as described by Stass and Dalhoff [12].

RESULTS

Spontaneous Emergence of Resistance

Spontaneous mutation rates differed between moxifloxacin and BAY y 3118, the structures of which are shown in figure 1. By use of an inoculum of $8 \times 10^{11}$ cfu/mL, the mutation rate toward moxifloxacin resistance was $6 \times 10^{-11}$, and the rate toward BAY y 3118 resistance was $4.7 \times 10^{-7}$. Emergence of multistep resistance was also different for the 2 quinolones tested (figure 2). Exposure to 0.5 times their MICs for 5 days resulted in a much less pronounced increase in MICs for moxifloxacin than for BAY y 3118.

Lack of Emergence of Resistance to Moxifloxacin during In Vitro Exposure to Fluctuating Concentrations

In vitro simulation of human moxifloxacin serum concentrations resulted in a dose-dependent reduction in viable counts of S. aureus 12241 (MIC 0.25 µg/mL) and an elimination of the inoculum within 8–12 h (figure 3). This effect was independent of the strain of organism used: S. aureus 133 was killed more effectively than S. aureus 12241, resulting in an elimination of the inoculum within 6–8 h, and strains 25927 and 25894 were killed as effectively as strain 12241 (data not shown). Regrowth did not occur with any of the strains tested, and, consequently, resistance did not emerge. Exposure of the laboratory-generated multistep resistant mutants (MIC, 1 µg/mL) to a 400-mg moxifloxacin dose ($C_{\text{max}} = 2$ mg/L) resulted in a bacteriostatic effect, but resistance also did not emerge in this setting (data not shown).

Emergence of Resistance to BAY y 3118 during Exposure to Fluctuating Concentrations

Exposure of S. aureus 133 to fluctuating concentrations of BAY y 3118 simulating human serum concentrations after a single oral dose of 200 mg resulted in a rapid elimination of the inoculum within 8 h, and regrowth did not occur.

However, S. aureus 25927 and S. aureus 25894 were marginally affected by a single oral dose of BAY y 3118. After a rapid initial reduction of viable counts by 2.5 to 3 logs within 2 h, bacteria regrew rapidly, and viable counts at 24 h were not significantly different from those of the drug-free controls (data not shown). Therefore, S. aureus 25927 and S. aureus 25894 were exposed to fluctuating BAY y 3118 concentrations simulating a twice-daily dosing regimen. Under these experimental conditions, regrowth was not as rapid and marked as described above, but regrowth still did occur; at 24 h, viable counts of S. aureus 25927 and S. aureus 25894 amounted to $3.4 \times 10^9$ and $9.8 \times 10^9$ cfu/mL, respectively. At 84 h, bacterial titers were $6.1 \times 10^9$ and $683.4 \times 10^9$ cfu/mL, respectively.

In parallel to a continuous increase in total viable counts of S. aureus 25927 and S. aureus 25897, resistance emerged rapidly.
Exposure of both *S. aureus* strains to fluctuating BAY y 3118 concentrations simulating a regimen of 200 mg twice daily caused a rapid and extensive selection of resistance, independent from the MICs of the test strains (figures 4 and 5). Resistance emerged within 6 h after commencement of exposure to BAY y 3118 and continued to increase throughout the incubation period.

**Lack of Emergence of Resistance to Moxifloxacin In Vivo**

**Pharmacokinetics.** After an oral dose of moxifloxacin of 100 mg/kg/day, mean maximum exudate concentrations of 1.2 mg/L (SD ± 0.42 μg/mL) were recorded 2 h after administration; concentrations declined, with a \( t_{1/2} \) of 5.2 h (SD ± 0.5 h). The pouch exudate concentrations of moxifloxacin were linearly proportional to the doses administered.

After an oral dose of 100 mg/kg/day of BAY y 3118, exudate concentrations peaked at 1.7 h after administration; the mean maximal pouch exudate concentration was 1.6 mg/L (SD ± 0.37 mg/L); the \( t_{1/2} \) was 4.9 h (SD ± 0.4 h). The pouch exudate concentrations of BAY y 3118 were also linearly dose proportional.

**Efficacy.** Moxifloxacin treatment (100 mg/kg) of animals 1 h after infection resulted in an elimination of *S. aureus* 133 wild type from the pouch exudate within 3 days (figure 6). Delaying the start of moxifloxacin therapy for 24 h still resulted in a rapid, although less marked, reduction of viable counts (figure 6). Because of this rapid reduction of viable counts, animals were treated with a lower dose (50 mg/kg), and even at this lower dose, the viable counts fell by nearly 3–4 logs (figure 7). Resistance did not occur. First-step mutants were also eliminated as rapidly as wild-type strains without resistance emerging. The multistep resistant mutant was largely unaffected by a 50-mg/kg dose of moxifloxacin; however, the MICs were the same at the end of treatment as at day 0.

Viable counts of *S. aureus* 133 wild type declined rapidly within the focus of infection on treatment with 100 mg/kg/day of BAY y 3118; the pouch exudate became sterile within 3 days. Treatment of animals with 50 mg/kg/day of BAY y 3118 was almost ineffective. Total cfu/mL of exudate at 24 h was almost as high in the treated animals as in the untreated controls (7.8 log cfu/mL vs. 8.24 log cfu/mL). All isolates were resistant against BAY y 3118 (data not shown). In the untreated control animals, the wild type and mutants of *S. aureus* all grew equally well in the pouch fluid.

**DISCUSSION**

The purpose of this study was to assess whether the presence of a methoxy group at the C-8 position of the fluoroquinolone structure confers an advantage in terms of decreased sensitivity to resistance development compared with the presence of a chlorine substituent at this position. Moxifloxacin and BAY y 3118 only differ from each other at the C-8 position, moxifloxacin carrying a methoxy substituent and BAY y 3118 a chlorine substituent.

This single substitution significantly lowered the propensity for mutant selection—that is, the methoxy moiety at C-8 selected mutants much less frequently than did the chlorine moiety. The in vitro spontaneous mutation frequencies were \( 6 \times 10^{-11} \) for moxifloxacin and \( 4 \times 10^{-7} \) for BAY y 3118. Moreover, multistep resistance developed rapidly against BAY y 3118 (>16-fold rise), but much more slowly against moxifloxacin (2-
fold rise). The 1-compartment in vitro model, used to simulate human serum concentrations of both drugs, showed a rapid and dose-dependent reduction in \(S.\) aureus colonies for moxifloxacin, with elimination of the inoculum by 8–12 h and no regrowth and consequently no resistance selection.

The same was not true for BAY y 3118, which was significantly less bactericidal and caused rapid resistance selection (within 2–6 h of BAY y 3118 exposure). Moreover, the rat pouch model further corroborated these observations and demonstrated no resistance to moxifloxacin in an in vivo model, whereas resistance to BAY y 3118 developed in this model after 24 h. The results of the in vivo study demonstrate that first-step mutants of \(S.\) aureus were eliminated as effectively from the focus of infection as was the wild-type strain.

Although the multistep-resistant strain was not significantly affected by moxifloxacin treatment, resistance did not develop either. The pronounced in vivo activity of moxifloxacin and the lack of resistance development is especially notable because the mean maximal pouch exudate concentrations were 50% less than those in serum of healthy human volunteers after the oral administration of a 400-mg dose. Furthermore, pouch exudate concentrations declined twice as rapidly as human serum levels, so that exposure of the infecting organism was 3.5- to 5-fold lower than in humans. These data thus support previous studies that link a lower propensity for the development of resistance to the structure of the antimicrobial.

Indeed, Drlica et al. [13] recently showed that quinolones with a methoxy moiety at the C-8 position were less affected by either DNA gyrase or topoisomerase IV changes. The results of this study also demonstrate that the C-8 methoxy group in moxifloxacin enhances its bactericidal activity against \(S.\) aureus; moxifloxacin was more potent than the chlorine derivative BAY y 3118. These results are well in agreement with published data: in liquid media as well as in a macrophage system, C-8 methoxy fluoroquinolones were found to be more bactericidal against \(Mycobacterium\) tuberculosis than their C-8 hydrogen-substituted counterparts [14]. Similarly, the C-8 methoxy group greatly enhanced the bactericidal and bacteriostatic activities of fluoroquinolones against a set of isogenic quinolone-resistant mutants of \(Escherichia\) coli [15] and lowered the mutant-preventing concentration [16]. Moxifloxacin and gatifloxacin are currently the only approved 8-methoxy fluoroquinolones.

In addition to the importance of the C-8 position, Pestova et al. [17] have shown that modification of the C-7 group is...
also important in preventing resistance. They found that a large, hydrophobic moiety at the C-7 reduces the ability of the bacterium to efflux the compound across its cell wall. Moxifloxacin and trovafloxacin have a diazobicyclo and an azo group, respectively, at C-7, both of which fit this profile.

Previous studies demonstrated that fluoroquinolone resistance development in *Streptococcus pneumoniae* was dissociated as well; resistance against moxifloxacin developed much less frequently and slower than against ciprofloxacin [18]. However, BAY y 3118 was not studied in the S. pneumoniae model. Clearly, as new fluoroquinolones are designed and evaluated, it is appropriate to consider the extent to which the structure of the antimicrobial impinges on both its antibacterial activity and the potential for susceptible strains to develop resistance to the new agent. For moxifloxacin, both of these aspects have been studied: moxifloxacin’s structure confers a potent activity against a wide range of respiratory tract pathogens, including gram-negative, gram-positive, anaerobic, and atypical bacteria [18–20], although the data presented here demonstrate that the molecular entity selected for the C-8 position of a fluoroquinolone enhances that agent’s lowered propensity for resistance.

It is important to recognize that the propensity for resistance development is dissociated among quinolones [21], even if those agents appear to be equipotent in terms of their MICs. Moxifloxacin integrates potent antibacterial activity with a diminished propensity for mutant selection.

References

13. Drlica K. Refining the fluoroquinolones: basic efforts to understand quinolone biology may prolong the public health value of these widely used antibacterial agents. ASM News 1999;65:410–5.