Fluoroquinolone Resistance in Anaerobic Bacteria following Exposure to Levofloxacin, Trovafloxacin, and Sparfloxacin in an In Vitro Pharmacodynamic Model

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Received 24 February 2000/Returned for modification 4 July 2000/Accepted 18 April 2001

This investigation explored pharmacodynamic characteristics of fluoroquinolones against Bacteroides thetaiotamicron and the potential for development of resistance. An in vitro model was used to generate kill curves with three fluoroquinolones at various area under the concentration-time curve (AUC)/MIC ratios. Concentration-independent killing was observed. Increases in MICs were noted following exposure to fluoroquinolones at AUC/MIC ratios of 6 to 14.

We have previously reported fluoroquinolone resistance in clinical and ATCC strains of Bacteroides fragilis while conducting experiments with fluoroquinolones in an in vitro pharmacodynamic model (8; M. L. Peterson, L. B. Hovde, D. H. Wright, A. D. Hoang, and J. D. Kotschaefer, Programs Abstr. 38th Intersci. Conf. Antimicrob. Agents Chemother., abstr. E-32, p. 176–177, 1998). Initially, while performing studies with trovafloxaclin, sparfloxacin, clinafloxacin, levofloxacin, and ciprofloxacin against B. fragilis (ATCC 23745), bacterial resistance was produced following a single exposure to sparfloxacin (8). MICs following exposure to the fluoroquinolones were all within a one-tube dilution of the preexposure MICs, with the exception of sparflacin, which demonstrated an eightfold increase. Serial passage of the resistant B. fragilis over 10 days demonstrated no change in MICs, indicating stable fluoroquinolone resistance.

Independent investigations exploring optimal pharmacodynamic parameters for fluoroquinolones in the treatment of anaerobic infections produced antimicrobial resistance in other strains of B. fragilis following exposure to trovafloxacin and levofloxacin (Peterson et al., 38th ICAAC). Following utilization of area under the concentration-time curve (AUC)/MIC ratios of 10 and 50, B. fragilis strain ATCC 25285 and B. fragilis clinical isolate M97-117 exhibited 4- to 32-fold increases in postexposure MICs of the drugs. Again, stable fluoroquinolone resistance was demonstrated by the maintenance of elevated MICs after serial passage of resistant isolates for seven consecutive days.

The purpose of this study was to investigate the potential of other organisms in the B. fragilis group to develop stable resistance in vitro after exposure to levofloxacin, trovafloxacin, and sparflacin and to further explore the pharmacodynamics of fluoroquinolones against anaerobic bacteria.

(This work was presented in part at the 39th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, Calif., 26 to 29 September 1999.)

Stock solutions of trovafloxacin (Pfizer Inc., Groton, Conn.), levofloxacin (R. W. Johnson Pharmaceutical Research Institute, Spring House, Pa.), and sparflacin (Rhone-Poulenc Rorer, Collegrove, Pa.) were prepared according to the manufacturer’s instruction and frozen at −80°C until needed. One strain of Bacteroides thetaiotamicron ATCC 29741 was utilized for all experiments. The MICs of trovafloxacin, levofloxacin, and sparflacin were determined by broth microdilution techniques in accordance with NCCLS guidelines prior to antibiotic exposure (7). Anaerobe Broth MIC (Difco Laboratories, Detroit, Mich.) was used for all susceptibility testing. MICs were determined in triplicate using an inoculum size of between 105 to 106 CFU/ml. The modal MICs were used for determining AUC/MIC ratios in all experiments. Quality control was monitored using Pseudomonas aeruginosa ATCC 27853. Acceptable ranges were those determined by NCCLS.

The in vitro pharmacodynamic system and methodology, previously described by Peterson et al., were used to conduct all-time-kill experiments (8). Antibiotic half-lives of 8, 10, and 18 h for levofloxacin, trovafloxacin, and sparflacin, respectively, were simulated in the model. Peak concentrations and AUCs for each fluoroquinolone were chosen to reflect values obtainable with common clinical use. Supra- and subtherapeutic concentrations were then selected to allow variation in AUC/MIC ratios (1, 5, 10, 50, and 150). An anaerobic environment was created by placing the pharmacodynamic system within a Bactron IV anaerobic chamber (Anaerobe Systems, Morgan Hill, Calif.). Each experiment was performed in duplicate for a duration of 24 h. Evaluation of fluoroquinolone peak concentrations was conducted by obtaining 1-ml samples at 1, 6 (sparflacin only), 8 (levofloxacin and trovafloxacin only), and 24 h. A previously validated high-performance liquid chromatography assay was used for concentration determination (10).

The development of fluoroquinolone resistance was assessed by determining MICs for colonies obtained from the 24-h postantibiotic exposure time-point plates. A greater than one-
and 293.

where the actual AUC/MIC ratios produced were 1, 6, 14, 75, targeted values (Table 1), with the exception of levofloxacin, macrokinetic profiles generated in the model were similar to the derived quinolone concentrations. The fluoroquinolone pharmacokinetic data were based on high-performance liquid chromatography 8, and 2 mg/liter, respectively. Experimental pharmacokinetic experiment to the time of maximal reduction in the log10 CFU/ml. Statistical significance was defined as a lack of overlap in the 95% confidence limits generated from the slope of each bacterium stability of resistance, were done in accordance with NCCLS guidelines (7). To confirm stability of resistance, B. thetaiotaomicron bacteria for which drug MICs were elevated were consecutively passed onto antibiotic-free anaerobic blood agar plates for 10 days. MIC testing was performed on days 1, 3, 7, and 10.

Time-kill curves were analyzed for the rate and extent of bacterial killing. Time to 3-log kill, or time to 99.9% reduction of the initial inocula, and the rate of reduction were determined by linear regression using GraphPad Prism 3.0 (GraphPad Software, Inc., San Diego, Calif.). The rate of killing was defined as the slope of the killing curve from the start of the experiment to the time of maximal reduction in the log10 CFU/ml. Statistical significance was defined as a lack of overlap in the 95% confidence limits generated from the slope of each bacterial kill-curve experiment. The extent of bacterial killing was assessed by the presence or absence of regrowth at 24 h. AUC from 0 to 24 h/MIC ratios were verified from drug concentration analysis and prerun MIC data to evaluate the relationship between the pharmacodynamic parameter, antibacterial effect, and emergence of resistance. The peak concentration, minimum concentration, and half-life were calculated by standard noncompartmental pharmacokinetic equations (9). The AUC from 0 to 24 h was calculated by the trapezoidal method.

The MICs of trovafloxacin, levofloxacin, and sparfloxacin against B. thetaiotaomicron prior to antibiotic exposure were 1, 8, and 2 mg/liter, respectively. Experimental pharmacokinetic data were based on high-performance liquid chromatography derived quinolone concentrations. The fluoroquinolone pharmacokinetic profiles generated in the model were similar to the targeted values (Table 1), with the exception of levofloxacin, where the actual AUC/MIC ratios produced were 1, 6, 14, 75, and 293.

Time-kill curves for all three fluoroquinolones against B. thetaiotaomicron are presented in Fig. 1 with pharmacodynamic results summarized in Table 1. All AUC/MIC ratios of ≥11 produced a 3-log10 kill by 14 h. For levofloxacin, no significant difference in the rate of kill was seen between AUC/MIC ratios of 6 and 75. The highest rate of kill occurred at an AUC/MIC ratio of 14, which was significantly faster than at all other ratios, while the lowest rate of kill was seen at an AUC/MIC ratio of 293. An AUC/MIC ratio of 1 simulated the growth control experiment. Regrowth occurred at some point after 12 h at AUC/MIC ratios of 6 and 14. No regrowth was seen with AUC/MIC ratios of 75 and 293. Exposure to sparfloxacin at AUC/MIC ratios of 12, 53, and 143 produced no significant differences in rates of kill. An AUC/MIC ratio of 1 simulated the growth control experiment, while the AUC/MIC ratio of 4 had solely a static effect on the viability of the organism. Regrowth occurred with sparfloxacin only at an AUC/MIC ratio of 12. It is interesting that a paradoxical effect occurred with both levofloxacin and sparfloxacin with increasing AUC/MIC ratios, as the slowest rate of kill was seen at the highest ratios. This difference was not significant for sparfloxacin, however. With trovafloxacin, no correlation could be seen between increasing AUC/MIC ratios and rate of kill once an AUC/MIC ratio of 11 was achieved. Again an AUC/MIC ratio of 1 simulated a growth control, while an AUC/MIC ratio of 5 produced a static effect. Regrowth occurred with trovafloxacin only at an AUC/MIC ratio of 11.

Table 1 depicts postexposure MICs for colonies isolated on the 24-h blood agar plates. MICs increased by fourfold following exposure to levofloxacin at AUC/MIC ratios of 6 and 14, while MICs increased by four- to eightfold following exposure to trovafloxacin and sparfloxacin at AUC/MIC ratios of 11 and 12, respectively. After serial passage for ten days onto antibiotic-free anaerobic blood agar plates, MICs of the three fluoroquinolones remained elevated, confirming stable resistance. MICs of all other 24-h sample colonies selected for evaluation

<table>
<thead>
<tr>
<th>Quinolone (MIC [mg/liter])</th>
<th>Peak (mg/liter)</th>
<th>AUC</th>
<th>AUC/MIC ratio</th>
<th>T3K (h)*</th>
<th>Slope (±SD)</th>
<th>95% confidence interval</th>
<th>r²</th>
<th>Bacterial regrowth</th>
<th>Post-fluoroquinolone exposure MIC (mg/liter)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levofloxacin (8)</td>
<td>0.8</td>
<td>8</td>
<td>1/N/A</td>
<td>11.4</td>
<td>−0.279 ± 0.017</td>
<td>−0.316 to −0.241</td>
<td>0.95</td>
<td>Yes</td>
<td>8 1 2</td>
</tr>
<tr>
<td></td>
<td>6.4</td>
<td>48</td>
<td>6</td>
<td>11.4</td>
<td>−0.379 ± 0.034</td>
<td>−0.452 to −0.306</td>
<td>0.90</td>
<td>Yes</td>
<td>32 4 8</td>
</tr>
<tr>
<td></td>
<td>10.4</td>
<td>112</td>
<td>14</td>
<td>6.8</td>
<td>−0.300 ± 0.013</td>
<td>−0.329 to −0.272</td>
<td>0.97</td>
<td>No</td>
<td>8 1 2</td>
</tr>
<tr>
<td></td>
<td>63.2</td>
<td>600</td>
<td>75</td>
<td>14.3</td>
<td>−0.235 ± 0.013</td>
<td>−0.263 to −0.208</td>
<td>0.96</td>
<td>No</td>
<td>8 1 2</td>
</tr>
<tr>
<td></td>
<td>276.0</td>
<td>2,344</td>
<td>293</td>
<td>14.3</td>
<td>−0.235 ± 0.013</td>
<td>−0.263 to −0.208</td>
<td>0.96</td>
<td>No</td>
<td>8 1 2</td>
</tr>
<tr>
<td>Sparfloxacin (2)</td>
<td>0.2</td>
<td>2</td>
<td>1/N/A</td>
<td>10.7</td>
<td>−0.315 ± 0.025</td>
<td>−0.367 to −0.262</td>
<td>0.92</td>
<td>Yes</td>
<td>64 8 16</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>8</td>
<td>4</td>
<td>5.9</td>
<td>−0.370 ± 0.034</td>
<td>−0.442 to −0.297</td>
<td>0.90</td>
<td>No</td>
<td>8 1 2</td>
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<tr>
<td></td>
<td>1.8</td>
<td>24</td>
<td>12</td>
<td>12.1</td>
<td>−0.277 ± 0.011</td>
<td>−0.300 to −0.253</td>
<td>0.98</td>
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<td>8 1 2</td>
</tr>
<tr>
<td></td>
<td>6.8</td>
<td>106</td>
<td>53</td>
<td>12.1</td>
<td>−0.277 ± 0.011</td>
<td>−0.300 to −0.253</td>
<td>0.98</td>
<td>No</td>
<td>8 1 2</td>
</tr>
<tr>
<td></td>
<td>17.2</td>
<td>286</td>
<td>143</td>
<td>12.1</td>
<td>−0.277 ± 0.011</td>
<td>−0.300 to −0.253</td>
<td>0.98</td>
<td>No</td>
<td>8 1 2</td>
</tr>
<tr>
<td>Trovafloxacin (1)</td>
<td>0.1</td>
<td>1</td>
<td>1/N/A</td>
<td>10.7</td>
<td>−0.358 ± 0.025</td>
<td>−0.412 to −0.304</td>
<td>0.94</td>
<td>Yes</td>
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</tr>
<tr>
<td></td>
<td>0.4</td>
<td>4</td>
<td>4/N/A</td>
<td>10.7</td>
<td>−0.358 ± 0.025</td>
<td>−0.412 to −0.304</td>
<td>0.94</td>
<td>Yes</td>
<td>32 4 16</td>
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<tr>
<td></td>
<td>0.9</td>
<td>11</td>
<td>11</td>
<td>10.2</td>
<td>−0.295 ± 0.009</td>
<td>−0.315 to −0.276</td>
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<tr>
<td></td>
<td>4.3</td>
<td>56</td>
<td>56</td>
<td>10.2</td>
<td>−0.295 ± 0.009</td>
<td>−0.315 to −0.276</td>
<td>0.99</td>
<td>No</td>
<td>8 1 2</td>
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<tr>
<td></td>
<td>11.4</td>
<td>138</td>
<td>138</td>
<td>6.0</td>
<td>−0.331 ± 0.030</td>
<td>−0.395 to −0.267</td>
<td>0.90</td>
<td>No</td>
<td>8 1 2</td>
</tr>
</tbody>
</table>

* T3K, time to 3-log kill.

b N/A, not achieved.

Levo, levofloxacin; Spar, sparfloxacin; Trova, trovafloxacin.
FIG. 1. Activity of levofloxacin at AUC/MIC ratios of 1 (*), 6 (●), 14 (■), 75 (●), and 293 (●) (A); activity of sparfloxacin at AUC/MIC ratios of 1 (*), 4 (●), 12 (■), 53 (●), and 143 (●) (B); and activity of trovafloxacin at AUC/MIC ratios of 1 (*), 4 (●), 11 (■), 56 (●), and 138 (●) (C) against B. thetaiotamicron 29741. Growth controls are represented by (X).
remained within a one-tube dilution of preexposure MICs. Suggested MIC breakpoints for susceptibility to fluoroquinolones of Bacteroides spp. are as follows: sensitive, ≤2 mg/liter; intermediate, 4 mg/liter; and resistant, ≥8 mg/liter (5; Trovan [trovafloxacin] package insert, Pfizer Inc.). According to preantibiotic-exposure MICs, B. thetaiotamicron is sensitive to both trovafloxacin and sparfloxacin, while the organism is already resistant to levofloxacin. After antibiotic exposure in the model, however, the increase in MICs renders B. thetaiotamicron either intermediate or resistant to trovafloxacin and sparfloxacin.

We chose to assess the antimicrobial effect of fluoroquinolones by measuring the rate and extent of bacterial killing. Although somewhat academic, since killing 99.9% of bacteria in 6 h versus killing in 12 h likely has no clinical significance, such measurements allow us to determine pharmacodynamic parameters that best predict antimicrobial efficacy and thereby influence appropriate dosing of the drug to achieve maximum benefit without promoting resistance. Since little data currently exists evaluating the pharmacodynamics of fluoroquinolones against anaerobes, we deem this information vital to the utility of this class in anaerobic infections.

Data from this study suggest that fluoroquinolones kill B. thetaiotamicron in a concentration-independent fashion, since no correlation could be made between increasing AUC/MIC ratios and the rate of kill once an AUC/MIC ratio of 11 was obtained. In our analysis, AUC/MIC ratios of >14, however, were necessary to prevent the occurrence of regrowth and the development of resistance. Retrospectively, selecting AUC/MIC ratios above 10 and below 50 would have allowed us to delineate a more definitive breakpoint. Although the AUC/MIC ratio does not appear to be an inherent predictor of the antimicrobial efficacy of fluoroquinolones against anaerobic bacteria, the ratios do appear to predict the development of resistance. Clinical implications are unclear; however, depending on the fluoroquinolone utilized, the MIC for the bacteria, and the site of infection, AUC/MIC ratios between 6 and 53 are feasible with typical dosing regimens. Conventional parenteral doses of alatrofloxacin of 200 to 300 mg/day typically produce AUC/MIC ratios of ≥30, assuming the MIC at which 90% of B. thetaiotamicron bacteria is inhibited is 1 mg/liter (Trovan package, insert, Pfizer Inc.). The trovafloxacin concentrations required to produce AUC/MIC ratios of less than 14 fall well below concentrations obtained from conventional dosing strategies, suggesting that with proper dosing, the appearance of fluoroquinolone-resistant B. thetaiotamicron following exposure to trovafloxacin may be prevented. On the contrary, conventional dosing regimens for both levofloxacin (500 mg/day) and sparfloxacin (200 mg/day) produce AUC/MIC ratios of <14 (Levaquin [levofloxacin] package insert, Ortho-McNeil Pharmaceutical, Inc.; Zagam [sparfloxacin] package insert, Rhone-Poulenc Rorer Pharmaceuticals Inc.) due to higher MICs in our study, 8 and 2 mg/liter, respectively, thereby making the development of resistant B. thetaiotamicron more likely.

Against B. fragilis, however, the breakpoint for the production of bacterial resistance was associated with AUC/MIC ratios of ≤50 (Peterson et al., 38th ICAAC). Again, conventional dosing schemes of levofloxacin and sparfloxacin fall within this range due to higher MICs (range, 1 to 4 mg/liter) (1, 4).

Trovafloxacin performance, however, would be heavily dependent upon the MIC for the organism. MICs of trovafloxacin for B. fragilis range from 0.125 to 1 mg/liter (1, 3, 4). With AUCs obtained from typical dosing regimens, organisms for which MICs fall below 1 mg/liter are likely to remain susceptible following exposure to trovafloxacin. On the contrary, if the MIC for the organism is 1 mg/liter, AUC/MIC ratios can be as low as 30 when using the 200-mg dose. Increasing the dose to 300 mg raises the AUC/MIC ratio near the breakpoint of 50, thereby highlighting the need to use the higher dose.

We noted that fluoroquinolone resistance was found only in experiments where a 3-log_{10} kill was followed by significant regrowth by 24 h. However, resistant populations may have been present in our 24-h cultures where no kill or less efficient kill occurred (i.e., AUC/MIC ratios of ≤4). Our MIC methodology, using inocula of 10^5 to 10^6 CFU/ml, simply was not designed to detect this occurrence, as mutation frequencies have previously been shown to be ≤6.4 × 10^{-9} (8). We are unsure, however, what the clinical significance of this finding would be. Since the in vitro system is devoid of host defense mechanisms, if the initial bacterial burden is reduced by 99.9%, a functioning immune system could get rid of the infection and prevent the survival of mutant subpopulations. Furthermore, subsequent doses may contribute to the overall antimicrobial effect and thereby influence the infectious process. Since only single-dose experiments were performed in this study, no such speculation can be made.

With in vitro data at hand, however, of particular interest would be the extrapolation of these findings into the clinical setting. Investigations have been performed determining the frequency of antimicrobial resistance in human fecal flora (2, 6), but to our knowledge, no studies have compared the MICs of fluoroquinolones for anaerobic fecal bacteria both prior to and following therapy. Accordingly, the prevalence of anaerobic resistance may be grossly underreported and unappreciated by clinicians, thereby highlighting the necessity of clinical investigations to determine the proper dosing of fluoroquinolones to prevent resistance. Recently, Gustafson et al. reported decreased susceptibility of anaerobic gram-negative bacteria to trovafloxacin received from across the United States from April 1998 to March 1999 (D. R. Gustafson, L. M. Sloan, and J. E. Rosenblatt, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 905, p. 218, 1999). While the specific reasons for these changes are not known, the authors suggest that the widespread use of fluoroquinolones may be a factor. Our opinion is that while the effectiveness of fluoroquinolone therapy in the management of upper and lower respiratory tract infections, urinary tract infections, and skin and soft tissue infections is not in question, their subsequent effect on other bacterial reservoirs has not been carefully elucidated. Due to their high bioavailability and large volume of distribution, fluoroquinolones are likely impacting organisms other than the intended species. Levofloxacin and sparfloxacin, while both excellent agents against gram-positive and gram-negative pathogens, possess only marginal activity against anaerobes at typical therapeutic concentrations. Although it is unlikely that either agent would be used to treat an anaerobic infection, their use in the treatment of gram-positive and gram-negative infections could promote stable fluoroquinolone resistance in anaerobic organisms. Patients treated with oral
fluoroquinolones for common ambulatory infections could be placed at risk should they contract an anaerobic infection at a later time that would be managed with a fluoroquinolone with anaerobic activity, such as trovafloxacin.

REFERENCES