In vitro activities of levofloxacin, gatifloxacin, moxifloxacin and garenoxacin against Bacteroides fragilis strains evaluated by kill kinetics

Reiner Schaumann,1† Eva Janssen,1† Matthias Funke,1 Catalina S. Stingu,1 Gelimer H. Genzel,1 Martin Janssen2 and Arne C. Rodloff1

1Institute for Medical Microbiology and Epidemiology of Infectious Diseases, University of Leipzig, Leipzig, Germany
2Department of Urology, University Clinics of Saarland, Homburg/Saar, Germany

This study was designed to investigate the killing activity of levofloxacin, gatifloxacin, moxifloxacin and garenoxacin against 12 Bacteroides fragilis strains by kill kinetics over time. MIC values were determined by Etest and by agar dilution. B. fragilis strains were divided according to their MIC values into two groups: one group with strains with MIC <8.0 μg ml⁻¹ and one group with strains with MIC ≥8.0 μg ml⁻¹. For kill kinetics over time, the strains with MIC <8.0 μg ml⁻¹ were incubated with the antibiotics at 0.5, 1, 2 and 4 times their MIC values. The strains with MIC ≥8.0 μg ml⁻¹ were incubated with 0.5, 1, 2, and 4 times the maximum achievable concentrations of the antibiotics in human plasma (Cmax). Among the strains with MIC <8.0 μg ml⁻¹ levofloxacin and gatifloxacin showed equal efficacy. The growth of the strains with MIC ≥8.0 μg ml⁻¹ was barely affected by levofloxacin, while gatifloxacin had bactericidal action when concentrations of 4×Cmax were used. Moxifloxacin was more effective against both groups of strains compared with levofloxacin and gatifloxacin. Garenoxacin was the most active agent against all strains investigated. Due to the varying in vitro activity of the quinolones against obligate anaerobes the treatment with quinolones of patients with intra-abdominal infections needs intensive scrutiny.

INTRODUCTION

Bacteroides fragilis is the most commonly isolated anaerobic pathogen (Wexler, 2007). Furthermore, members of the B. fragilis group, especially B. fragilis and B. thetaiotaomicron, are more resistant to antimicrobial agents than most other anaerobic bacteria. Several studies indicate increasing resistance rates of B. fragilis strains against commonly used antimicrobial agents such as cefoxitin, clindamycin and piperacillin/tazobactam (Betriu et al., 2008; Hedberg et al., 2003; Nagy et al., 2011). Quinolones have been used since 1964 for the treatment of urinary tract infections (Tillotson, 1996) with proven efficacy, since they are still recommended as the first-line therapy for complicated urinary tract infections, prostatitis and acute pyelonephritis (Fünfstück et al., 2012; Liu & Mulholland, 2005). Changes in the molecular structure of quinolones have led to the development of a group of quinolones with a wide range of possible indications (Schaumann & Rodloff, 2007). Naber & Adam (1998) classified the quinolones into four groups based on their activity and their possible range of indications. Newer quinolones are of growing importance in the treatment of anaerobic or mixed infections (Lubasch et al., 2000; Stein & Goldstein, 2006). However, the problem of increasing resistance rates also applies to quinolones. Golan et al. (2003) found that quinolone resistance has been increasing among Bacteroides since 1994. Recently, Nagy et al. (2011) reported a dramatic increase in resistance to cefoxitin, clindamycin and moxifloxacin, with resistance rates of 17.2 %, 32.4 % and 13.6 %, respectively, throughout Europe, with higher resistance rates for moxifloxacin in Scandinavian countries than in Mediterranean countries (Fille et al., 2006; Hedberg et al., 2003). Thus, the knowledge of resistance patterns is important for adequate prophylaxis and treatment of anaerobic or mixed aerobic and anaerobic infections. As studies employing kill kinetics are expensive in terms of time and cost, they are not likely to be performed in the routine laboratory. Therefore, the aim of the present study was to investigate the in vitro killing activity of four different quinolones against 12 B. fragilis strains evaluated by kill kinetics over time.

METHODS

Bacterial strains. Twelve B. fragilis isolates were selected from the institute’s stock. The RMA strains were kindly provided by E. J. C. Goldstein, R. M. Alden Research Lab, Culver City, California, USA.
The WAL strains were collected at the University Hospital of Wales, Cardiff, UK, and are isolates of an international anaerobe study tested at the Institute for Medical Microbiology and Epidemiology of Infectious Diseases, University of Leipzig, Germany. The strains were chosen due to their well-investigated characteristics in previous studies (Schaumann et al., 2004, 2005a,b, 2012).

Antimicrobial agents. Standard antibiotic powders of known activity were provided by the manufacturers as follows: levofloxacin, Aventis Pharma, Frankfurt/M., Germany; gatifloxacin, Grunenthal GmbH, Aachen, Germany; moxifloxacin, Bayer Health Care Pharma, Leverkusen, Germany; and garenoxacin, Bristol-Myers Squibb Company, Munich, Germany. Stock solutions were prepared and kept frozen at −70°C until the day of use.

Kill-kinetics medium. Brucella broth (Becton Dickinson) supplemented with vitamin K₁ (Sigma) and haemin (Serva Feinbiochemica) was used as the growth medium.

MIC determination. MIC values of levofloxacin, gatifloxacin and moxifloxacin for the B. fragilis strains were determined using Etest (AB Biodisk) according to the manufacturer’s instructions on supplemented Columbia agar with a final inoculum of 3 × 10⁸ c.f.u. ml⁻¹, as reported previously (Schaumann et al., 2005b). Columbia agar was supplemented with sheep blood (Oxoid), vitamin K₁ and haemin. MIC values of garenoxacin for the strains were determined using the agar dilution method on brucella agar (Oxoid) supplemented with vitamin K₁, haemin and defibrinated horse blood (Oxoid) with a final inoculum of 10⁵ c.f.u. per spot, as described previously for gemifloxacin (Kleinkauf et al., 2001).

Kill kinetics. B. fragilis strains were divided according to their MIC values into two groups: one group with strains with MIC <8.0 μg ml⁻¹ and one group with strains with MIC ≥8.0 μg ml⁻¹. For kill kinetics over time strains with MIC <8.0 μg ml⁻¹ were incubated with 0.5, 1, 2, and 4 times the MIC values over 24 h. Strains with MIC ≥8.0 μg ml⁻¹ were incubated with 0.5, 1, 2, and 4 times the maximum achievable concentrations (Cₘₐₓ). The maximal achievable peak plasma concentrations in humans were taken as Cₘₐₓ values. The following reported peak plasma concentrations were used as Cₘₐₓ for levofloxacin, 6 μg ml⁻¹ (Odenholt & Cars, 2006); for gatifloxacin, 3 μg ml⁻¹ and for moxifloxacin, 4 μg ml⁻¹ (Lubasch et al., 2000); and for garenoxacin, 5 μg ml⁻¹ (Gajjar et al., 2003).

One antibiotic-free growth control sample was run in parallel with each experiment. The turbidity of the initial inocula was adjusted to that of a 0.5 McFarland standard. The final inocula contained approx. 1.5 × 10⁷ c.f.u. ml⁻¹. At 0, 2, 4, 6, 12 and 24 h after incubation, aliquots were obtained and plated on supplemented Columbia agar. C.f.u. were counted after 48 h incubation at 37°C. The detection limit was 10² c.f.u. ml⁻¹. All experiments were performed in an anaerobic chamber (Heraeus) containing an atmosphere of 5% H₂, 15% CO₂ and 80% N₂.

Statistical analysis. For all strains and their respective antimicrobial agents, the mean value and standard deviation were calculated. Statistical analyses were performed using SPSS software. In those cases where the number of strains exceeded three, the paired-sample Wilcoxon signed-rank test was employed to identify significant differences. In each case, differences were calculated at t=6 h and t=24 h. A P-value <0.05 was considered to be significant.

RESULTS

The MIC values of the four antimicrobial agents for the tested B. fragilis strains are shown in Table 1. EUCAST provides no breakpoints for the antimicrobial agents and anaerobes tested here (EUCAST, 2012). In 2007 CLSI provided the following breakpoints for moxifloxacin and anaerobes: susceptible, ≤2 μg ml⁻¹; intermediate, 4 μg ml⁻¹; resistant, ≥8 μg ml⁻¹ (CLSI, 2007). Thus, a cut-off of ≥8.0 μg ml⁻¹ was chosen to separate the tested strains into a wild-type group (<8 μg ml⁻¹) and a resistant group (≥8 μg ml⁻¹). However, the CLSI breakpoint of ≥8 μg ml⁻¹ is higher than the available peak plasma concentrations of the quinolones tested here. Furthermore, the resistant strains showed MIC values higher than the Cₘₐₓ.

The pooled kill-kinetics curves for the B. fragilis strains with MIC <8 μg ml⁻¹ are shown in Fig. 1. Using 1 × MIC, only for garenoxacin was bactericidal activity obtained after 12 h. After 24 h, both garenoxacin and moxifloxacin showed bactericidal activity at concentrations of 1 × MIC. Levofloxacin and gatifloxacin showed bactericidal activity at concentrations of 2 × MIC or more. A significant difference was obtained for levofloxacin comparing 0.5 × MIC with 4 × MIC after 24 h incubation (P<0.05). After 6 h incubation, moxifloxacin, garenoxacin and gatifloxacin showed significant differences in killing rates comparing 1 × MIC

Table 1. MICs (μg ml⁻¹) of the B. fragilis strains tested

<table>
<thead>
<tr>
<th>B. fragilis strain</th>
<th>Levofloxacin</th>
<th>Gatifloxacin</th>
<th>Moxifloxacin</th>
<th>Garenoxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMA 0309</td>
<td>1</td>
<td>0.5</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>RMA 6791</td>
<td>1</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>RMA 5935</td>
<td>1</td>
<td>0.5</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>RMA 6600</td>
<td>2</td>
<td>0.5</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>RMA 5120</td>
<td>2</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>RMA 5081</td>
<td>2</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>WAL R 13054</td>
<td>2</td>
<td>0.5</td>
<td>0.5</td>
<td>2</td>
</tr>
<tr>
<td>WAL R 13054</td>
<td>2</td>
<td>0.5</td>
<td>0.5</td>
<td>2</td>
</tr>
<tr>
<td>RMA 5691</td>
<td>4</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>RMA 5138</td>
<td>4</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>WAL R 13174</td>
<td>4</td>
<td>1</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>WAL R 5798</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>WAL R 13267</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>8</td>
</tr>
</tbody>
</table>
and 4 × MIC (P<0.05). Furthermore, at 6 h and 24 h for moxifloxacin a significant reduction was seen comparing 0.5 × MIC and 1 × MIC or more. Garenoxacin showed a significant increase in antibacterial activity at 4 × MIC compared to 0.5 × MIC (P<0.05).

The pooled kill-kinetics curves for the B. fragilis strains with MIC values ≥8 µg ml⁻¹ are shown in Fig. 2. Levofloxacin did not show a reduction of more than 1 log step. Gatifloxacin showed significant bactericidal activity only at 4 × C_max. Bactericidal activity was obtained for moxifloxacin at 1 × C_max or more after 24 h. For garenoxacin already 0.5 × C_max resulted in bactericidal activity after 12 h.

**DISCUSSION**

The MIC value is a well-established and widely available parameter for the determination of susceptibility patterns of aerobic bacteria (Mueller et al., 2004). Many different methods, such as broth dilution, agar dilution and the Etest, are routinely used (Wilson & Huh, 1997). In contrast, susceptibility testing of anaerobes is not generally used (Hedberg et al., 2003; Nguyen et al., 2000). Reasons include the slow growth rate of anaerobes, the polymicrobial nature of anaerobic infections, the cost and complexity of testing methods, a lack of a widely accepted ‘gold standard’, and a belief in predictable resistance patterns among anaerobes (Fille et al., 2006; Hedberg et al., 2003; Nguyen et al., 2000). Kill-kinetics curves over time provide more information than the widely used MIC determination and allow a comparison of different antimicrobial concentrations, classes, and different substances of the same class (Mueller et al., 2004; Stratton et al., 1987).

In our study, the assessed kill kinetics correlated well with the MIC values of the strains tested when the organisms were susceptible to the respective antimicrobial agent. According to Naber & Adam (1998), levofloxacin belongs to group III of the quinolones. Gatifloxacin, moxifloxacin and garenoxacin are assigned to group IV, with improved activity against anaerobes (Naber & Adam, 1998). Compared to group IV of the quinolones, levofloxacin showed less bactericidal activity against the tested strains and notably did not affect the resistant strains at all. The first agent approved for clinical use of the group IV quinolones tested here was gatifloxacin (Hosaka et al., 1992).

A slightly better activity of gatifloxacin compared to levofloxacin, especially in the resistant strains, was observed. This underlines the results of clinical studies showing an advantage of gatifloxacin in the treatment of uncomplicated skin and soft tissue infections (Tarshis et al., 2001).
Moxifloxacin reaches almost the same concentration in peritoneal exudate and plasmal blood within 2 h after intravenous application: 3.61 \( \mu g \) ml\(^{-1}\) in plasma after 1 h versus 3.32 \( \mu g \) ml\(^{-1}\) in peritoneal exudate after 2 h (Stass et al., 2006). Since we have used serum \( C_{max} \) concentrations in previous studies (Schaumann et al., 2004, 2005a) these concentrations were also used in the present study, resulting in a better in vitro activity of moxifloxacin compared to levofloxacin or gatifloxacin against the anaerobes tested here. The report of Goldstein et al. (2011b) analysing results from four well-controlled complicated intra-abdominal infections trials, and the correlation with MIC and clinical efficacy data, support the conclusion that moxifloxacin is clinically efficacious in therapy of these infections. Clinical success with moxifloxacin was active against all the strains we tested, including strains for which the MIC value was 4 \( \mu g \) ml\(^{-1}\) and those for which it was >32 \( \mu g \) ml\(^{-1}\).

Garenoxacin proved to have the best in vitro activity compared to levofloxacin, gatifloxacin and moxifloxacin. Again this result correlates well with previous reports (Goldstein et al., 2011a; Schaumann & Rodloff, 2007). However, comparing our results to those of Schaumann et al. (2012), metronidazole turned out to be more efficient than the quinolones tested here, but metronidazole still needs a combination to cover aerobic bacteria in mixed infections.

Garenoxacin and gatifloxacin were either never licensed or were withdrawn due to severe side-effects in Europe and the USA (European Medicines Agency, 2007; Liu, 2010). Currently, garenoxacin is available for oral administration in Japan and has proven to be effective in the treatment of community-acquired pneumonia (Ohsaki et al., 2010; Tanigawara, et al., 2012). Gatifloxacin was recently investigated for the treatment of non-gonococcal urethritis (Hamasuna et al., 2011). Furthermore, Goldstein et al. (2011a) reported good activity of garenoxacin against isolates recovered from acute pelvic infections including anaerobes and concluded that this antibiotic could have potential utility in mixed aerobic/anaerobic infections.

In conclusion, due to the varying in vitro activity of the quinolones against obligate anaerobes the treatment with quinolones of patients with anaerobic or mixed aerobic/anaerobic infections needs intensive scrutiny.

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