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Bactericidal activity of gemifloxacin and other quinolones against *Streptococcus pneumoniae*

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This study compared the bactericidal activity of gemifloxacin (SB-265805) and a panel of test quinolones against two ciprofloxacin-resistant pneumococcal strains (*Streptococcus pneumoniae* 502226 and 503244) and one ciprofloxacin-sensitive strain (*S. pneumoniae* C3LN4). Activities were compared by calculating the bactericidal index of these agents. Gemifloxacin was found to be the most bactericidal quinolone tested against these strains. This finding confirms previous data indicating the superior *in vitro* activity of gemifloxacin against pneumococci, including ciprofloxacin-resistant strains. Although both ciprofloxacin-resistant strains tested had similar quinolone MICs, they differed considerably in their susceptibility to the bactericidal action of these agents. *S. pneumoniae* 502226 was more readily killed by quinolones than *S. pneumoniae* 503244 but, as would be expected, both were less susceptible than the ciprofloxacin-sensitive strain. Of the quinolones tested, trovafloxacin showed disproportionally poor activity against the ciprofloxacin-resistant strains even though potent activity was present against the ciprofloxacin-sensitive strain. These data highlight the importance of assessing quinolone bactericidal activity in addition to the MIC when evaluating new members of this antimicrobial class.

Introduction

Gemifloxacin is a novel quinolone with potent, broad-spectrum antibacterial activity. Preliminary studies suggest that gemifloxacin is more potent than other quinolones against the common respiratory tract pathogen *Streptococcus pneumoniae*.[1] Recent data also indicate that gemifloxacin retains this potency against pneumococci that are resistant to ciprofloxacin or trovafloxacin.[2]

In order to provide further data on the activity of gemifloxacin *in vitro*, this study investigated the bactericidal activity of gemifloxacin compared with ciprofloxacin, levofloxacin, grepafloxacin, moxifloxacin and trovafloxacin against the ciprofloxacin-resistant strains *S. pneumoniae* 502226 and 503244, and the ciprofloxacin-sensitive strain *S. pneumoniae* C3LN4. Bactericidal activity was compared by calculating the bactericidal index, a recently proposed method of measuring the bactericidal activity of antimicrobial agents.[3]

Materials and methods

Antimicrobial agents

Gemifloxacin, trovafloxacin, levofloxacin, ciprofloxacin and grepafloxacin were provided by SmithKline Beecham Pharmaceuticals (Harlow, UK). Moxifloxacin was provided by Bayer AG (Wuppertal, Germany). All quinolone solutions (2.5 mg/L) were freshly prepared on each day of the study.

Bacterial strains

This study used the following bacterial strains. *S. pneumoniae* C3LN4 is a ciprofloxacin-sensitive laboratory strain that has been used previously to assess the bactericidal activity of quinolones.[4] *S. pneumoniae* 502226 and 503244 are ciprofloxacin-resistant (MIC 16 mg/L) clinical isolates from the Alexander Project culture collection.[5] *S. pneumoniae* 502226 has amino acid changes Ser81→Phe in GyrA, Asp83→Asn in ParC and Ile460→Val in ParE,

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while *S. pneumoniae* 503244 has changes Ser81→Phe in GyrA, Ser79→Tyr and Ala189→Val in ParC and Ile460→Val in ParE.[6] The quinolone MICs for these strains are shown in Table I.

Organisms were stored at –70°C and subcultured on to Mueller–Hinton agar (Unipath, Basingstoke, UK) supplemented with 5% (v/v) saponin-lysed horse blood (Unipath) before use.

Bactericidal tests

The bactericidal activity of each quinolone was investigated at concentrations of 0.005–10 mg/L in Mueller–Hinton broth (Unipath) supplemented with 5% (v/v) saponin-lysed horse blood. Exponentially growing bacteria were used with an initial inoculum size of between 2.2×10^5 and 1.9×10^6 cfu/mL and incubated for 3 h at 37°C. After incubation, 1 mL samples were centrifuged and the bacterial pellets were resuspended in an equal volume of sterile broth. This washing step was repeated to prevent drug carryover during viable counting. Viable counts of these samples were made on solid agar by spiral plating, and plates were incubated for 48 h at 35°C. Experiments were carried out in duplicate, and the mean $\log_{10}$ change in viable count was calculated and plotted against drug concentration.

A bactericidal index was also calculated for the mean of duplicate bactericidal tests. This was done by plotting $\log_{10}$ reduction in viable count against $\log_{10}$ drug concentration and measuring the area under the curve of the bactericidal section of the graph using Fig.P software (Fig.P, Cambridge, UK) as described previously.[3] An example of this type of graph is shown in Figure 1. The AUC for the more potent quinolone (gemifloxacin) is greater than that for the weaker quinolone (ciprofloxacin).

Table I. Activity of test quinolones against *S. pneumoniae* strains C3LN4, 502226 and 503244

	MIC (mg/L)		
Quinolone	C3LN4	502226	503244
Gemifloxacin	0.06	0.5	0.5
Ciprofloxacin	2	16	16
Levofloxacin	1	16	16
Grepafloxacin	0.5	16	16
Moxifloxacin	0.12	2	4
Trovafloxacin	0.06	2	8

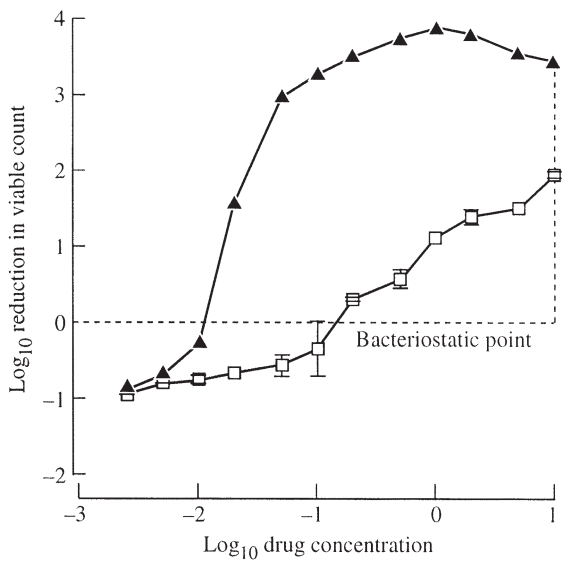

Figure 1. Graph of $\log_{10}$ reduction in viable count against $\log_{10}$ drug concentration used to calculate AUC for gemifloxacin and ciprofloxacin against *S. pneumoniae* C3LN4. ▲, gemifloxacin; □, ciprofloxacin.

MIC determination

MICs were determined by the NCCLS microbroth dilution method in Mueller–Hinton broth supplemented with 2% freeze–thaw lysed horse blood with an inoculum of approximately 10^5 cfu/mL.[7] Following inoculation, plates were incubated for 20–24 h at 35°C. MIC endpoints were read as the lowest concentration of antimicrobial that completely inhibited visible growth. Results were only accepted if MIC determinations for the control strain (*S. pneumoniae* ATCC 49619) were within the performance range.

Results

The bactericidal profiles for all six quinolones against the ciprofloxacin-sensitive *S. pneumoniae* C3LN4 are shown in Figure 2. It can be seen that gemifloxacin was the most bactericidal quinolone tested, producing, at its optimum bactericidal concentration, almost a 4 log reduction in viability after 3 h. All the test quinolones were considerably less bactericidal against the ciprofloxacin-resistant pneumococci (Figures 3 and 4). Nevertheless, gemifloxacin, at its optimum bactericidal concentration, produced almost a 3 log reduction in viability of *S. pneumoniae* 502226 (Figure 3). Against *S. pneumoniae* 503244, gemifloxacin at its optimum bactericidal concentration produced almost a 2 log reduction in viable count (Figure 4).

To quantify the differences in quinolone bactericidal activity shown in Figures 1–3, the bactericidal index was calculated for each bactericidal profile. The calculated bactericidal indexes for each test quinolone against the three pneumococci are shown in Table II. Against the quinolone-sensitive *S. pneumoniae* C3LN4 gemifloxacin produced the highest bactericidal index, as would be expected from the data shown in Figure 2. Gemifloxacin was 1.4 times as active as trovafloxacin, twice as active as

moxifloxacin and 2.4–4.9 times as active as grepafloxacin, levofloxacin or ciprofloxacin against *S. pneumoniae* C3LN4. All the test quinolones were less bactericidal against *S. pneumoniae* 502226 or *S. pneumoniae* 503244 than against *S. pneumoniae* C3LN4, as illustrated in Figures 2–4. Nevertheless, gemifloxacin still had a bactericidal index of 4.7 against *S. pneumoniae* 502226, which was either equal or superior to the bactericidal index of moxifloxacin, grepafloxacin, levofloxacin and ciprofloxacin against quinolone-sensitive *S. pneumoniae* C3LN4. Interestingly, trovafloxacin

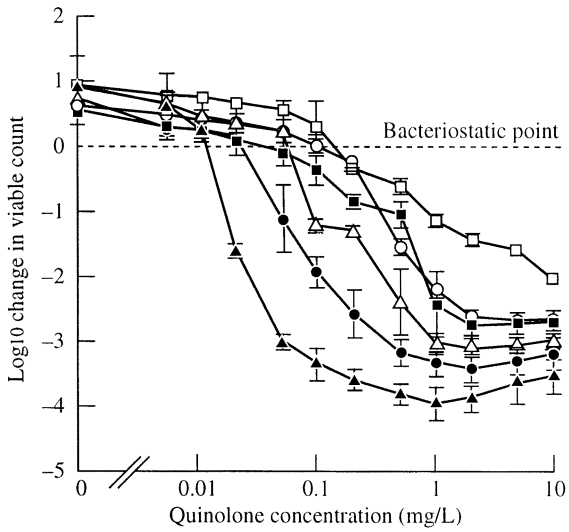

Figure 2. Bactericidal activity of quinolones against ciprofloxacin-sensitive *S. pneumoniae* C3LN4 after incubation for 3 h at 37°C (error bars represent the range of two determinations). ▲, gemifloxacin; △, moxifloxacin; ●, trovafloxacin; ○, levofloxacin; ■, grepafloxacin; □, ciprofloxacin.

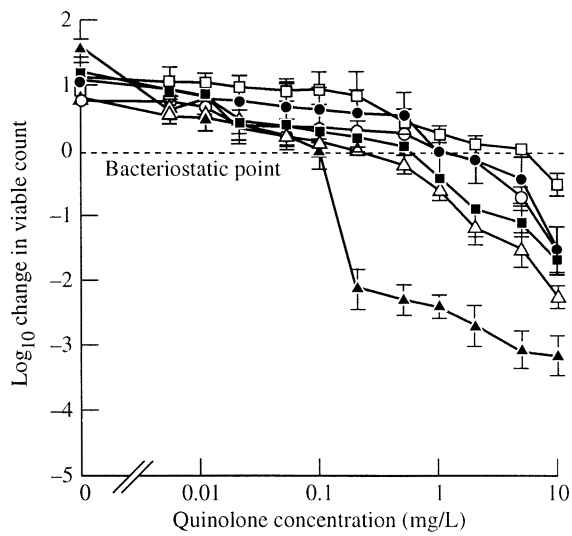

Figure 3. Bactericidal activity of quinolones against ciprofloxacin-resistant *S. pneumoniae* 502226 after incubation for 3 h at 37°C (error bars represent the range of two determinations). Symbols as in Figure 2.

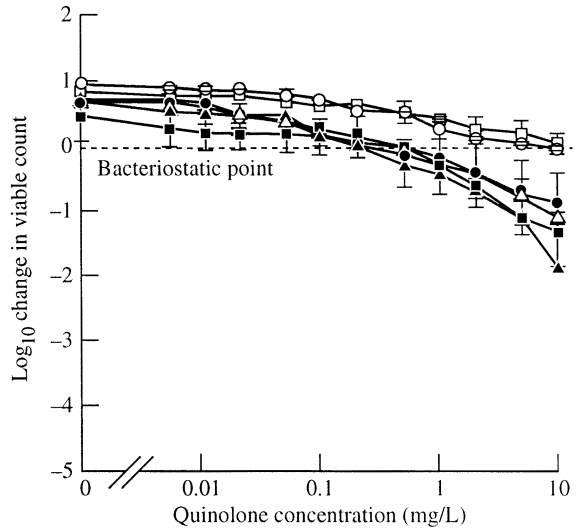

Figure 4. Bactericidal activity of quinolones against ciprofloxacin-resistant *S. pneumoniae* 503244 after incubation for 3 h at 37°C (error bars represent the range of two determinations). Symbols as in Figure 2.

Table II. Bactericidal index of test quinolones against *S. pneumoniae* strains C3LN4, 502226 and 503244

	Bactericidal index		
Quinolone	C3LN4	502226	503244
Gemifloxacin	9.3	4.7	1.0
Trovafloxacin	6.6	0.4	0.6
Moxifloxacin	5.0	1.4	0.6
Grepafloxacin	3.8	1.0	0.8
Levofloxacin	3.4	0.5	0
Ciprofloxacin	1.9	0.1	0

lost essentially all bactericidal activity (bactericidal index = 0.4) against ciprofloxacin-resistant *S. pneumoniae* 502226 despite being the second most potent quinolone against ciprofloxacin-sensitive *S. pneumoniae* C3LN4.

S. pneumoniae 503244 was less readily killed by the quinolones than *S. pneumoniae* 502226, even though the quinolone MICs for these two strains were very similar (Table II). Gemifloxacin was the most bactericidal quinolone tested against *S. pneumoniae* 503244 but, despite this, gemifloxacin was 4.7 times less active against *S. pneumoniae* 503244 than against *S. pneumoniae* 502226.

Discussion

This study found that gemifloxacin was the most bactericidal of all the quinolones tested against the three pneumococcal strains, which emphasizes the excellent activity of

this novel quinolone against *S. pneumoniae in vitro*. In addition, the data presented here confirm that gemifloxacin retains bactericidal activity as well as inhibitory activity against ciprofloxacin-resistant pneumococci.[2] This property was not shared by the other quinolones investigated. The two ciprofloxacin-resistant isolates, with similar MICs, were killed to quite different extents by the quinolones, including gemifloxacin. Recently, *in vitro* profiles of a number of quinolones against *S. pneumoniae* have also shown that bactericidal activity and post-antibiotic effect *in vitro* bear no relation to MIC.[8]

Further evidence that quinolone MIC and bactericidal potency are unrelated has been published previously for *Pseudomonas aeruginosa*.[9] In addition, a study in *Escherichia coli* found that pH and Mg^{2+} ions affect quinolone MIC and bactericidal activity quite differently.[10] These findings illustrate the importance of assessing the bactericidal activity as well as the MIC of quinolones.

A biochemical mechanism to explain the distinction between quinolone bactericidal activity and MIC has been proposed. According to this mechanism, the bacteriostatic activity of quinolones involves the formation of antimicrobial–enzyme–DNA complexes, while their bactericidal activity follows the release of lethal DNA breaks.[11] Thus, investigations relying merely on MIC can be misleading because these tests only measure non-lethal complex formation.

Acknowledgement

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