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The bactericidal activity of levofloxacin compared with ofloxacin, D-ofloxacin, ciprofloxacin, sparfloxacin and cefotaxime against *Streptococcus pneumoniae*

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The bactericidal activity of levofloxacin was compared with that of four other quinolones and one cephalosporin against four strains of *Streptococcus pneumoniae* with varying degrees of penicillin G susceptibility. Levofloxacin was found to be the most bactericidal quinolone at its optimum bactericidal concentration, followed by ofloxacin, ciprofloxacin, sparfloxacin and D-ofloxacin, in that order. There was no correlation between quinolone-susceptibility and penicillin-susceptibility with any quinolone tested. To allow a comparison between the bactericidal activity of the quinolones and cefotaxime to be made a bactericidal index was used.[1] Against two penicillin-sensitive pneumococci and a penicillin-intermediate pneumococcus, cefotaxime was the most potent antibacterial agent tested, followed by levofloxacin. However, against the penicillin-resistant pneumococcus, levofloxacin was considerably more potent than cefotaxime. The remaining quinolones tested were inferior to levofloxacin. Levofloxacin has enhanced activity against pneumococci compared with clinically available quinolones. In addition, levofloxacin may be the future quinolone of choice in the treatment of penicillin-resistant pneumococci.

Introduction

During the last 20 years an increase in penicillin-resistant pneumococci has occurred.[2] The first report of a penicillin-resistant strain of *Streptococcus pneumoniae* in the western world was from the USA in 1965.[3] A decade later, high-level penicillin-resistant pneumococci (MIC $\geqslant$ 2 mg/L) and multiple-drug-resistant pneumococci emerged in South Africa.[4,5] Penicillin-resistant pneumococci are now isolated worldwide, among the worst affected areas being South Africa, Spain and Eastern Europe.[6] It is therefore imperative to test alternative therapeutic compounds for activity against penicillin-resistant pneumococci. One such option is the use of newer fluoroquinolones, which although more commonly associated with the treatment of some Gram-negative bacterial infections may possess good antibacterial activity against Gram-positive bacteria.[7]

When bacteria are treated with a range of quinolone concentrations (a bactericidal profile), a biphasic dose–response normally occurs producing a single concentration of maximum kill known as the optimum bactericidal concentration or OBC.[8] Previously, the bactericidal activity of quinolones has been assessed by comparing OBCs and the percent survival at the OBC. However, this method may have its drawbacks.[1]

Levofloxacin is the antimicrobially active optical isomer of ofloxacin, whereas D-ofloxacin is the inactive isomer.[9] Levofloxacin is in use clinically in Japan and Hong Kong, but is not available elsewhere. Most studies have found levofloxacin to be twice as potent as ofloxacin.[10] Previous studies have indicated that levofloxacin may be of use against *S. pneumoniae* infections.[11,12] Sparfloxacin has also been shown to have good antipneumococcal activity.[6,13]

Therefore, in this study the bactericidal profiles for levofloxacin and sparfloxacin (as examples of fluoroquinolones whose introduction is anticipated shortly) and ofloxacin and ciprofloxacin (as established fluoroquinolones) were compared against four strains of *S. pneumoniae* with

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varying degrees of susceptibility to penicillin G. Cefotaxime was included in the study as an antipneumococcal third-generation cephalosporin. The inactive stereoisomer of ofloxacin, D-ofloxacin, was also investigated. The data were used to calculate bactericidal indices (BIs) for the drugs.[1]

Materials and methods

Bacterial strains

S. pneumoniae C3LN4 (penicillin G-susceptible (MIC 0.001 mg/L)) was selected for study because of its use in previous bactericidal tests of quinolones against pneumococci.[11,14] *S. pneumoniae* 269, *S. pneumoniae* 16000 and *S. pneumoniae* KPR are clinical isolates chosen as examples of penicillin-sensitive, penicillin-intermediate and penicillin-resistant strains, with penicillin G MICs of 0.007, 0.2 and 2 mg/L, respectively.

Antibacterial agents

The following antibacterial powders were used: cefotaxime, levofloxacin, D-ofloxacin, ofloxacin (Roussel UCLAF, Romainville, France), sparfloxacin (Rhône–Poulenc Rorer, Vitry sur Seine, France) and ciprofloxacin (Bayer UK, Newbury, UK). The drugs were diluted in 0.1 M sodium hydroxide at 2.5 mg/L and immediately diluted in sterile distilled water.

Determination of bactericidal activity (bactericidal profiles)

The bactericidal activity of each test drug was investigated by the method of Morrissey & Smith.[14] Briefly a range of drug concentrations between 0.05 and 90 mg/L was prepared in Oxoid nutrient broth No.2 (Unipath, Basingstoke, UK) containing 7% (v/v) laked horse blood (Unipath). Bacteria were inoculated to an initial inoculum of about 10^6 cfu/mL and incubated for 3 h at 37°C. After incubation, viable counts were made on solid blood agar. Experiments were carried out in triplicate and average results used.

Calculation of bactericidal indices

Results obtained from the bactericidal profiles were used to produce a BI for each drug against each bacterial strain.[1] The BI for each drug against each strain was calculated by plotting the logarithm of reduction in viability against the logarithm of drug concentration. The BI was taken as the AUC for the bactericidal portion of this plot up to the peak serum concentration of each drug (Table I).[1]

Results

The quinolone bactericidal profiles against *S. pneumoniae* C3LN4 are shown in Figure 1. All the test quinolones except D-ofloxacin displayed a biphasic dose response which is characteristic of quinolone bactericidal activity.[8] D-Ofloxacin may also have produced a biphasic dose response if higher drug concentrations had been tested. D-Ofloxacin was essentially inactive up to 90 mg/L. There was no statistically significant difference between the quinolone profiles obtained for *S. pneumoniae* C3LN4 and quinolone profiles obtained for the other strains tested (Pearson correlations between 0.89 and 0.99). These bactericidal profiles are not shown.

The OBC's for the quinolones tested (except for D-ofloxacin) and the reduction in log cfu obtained at each OBC are shown in Table II. All the quinolones possessed similar OBCs of 3 or 5 mg/L against the pneumococci. However, differences in bactericidal activity at the OBC occurred, as measured by the log decrease in bacterial viability. Levofloxacin was the most bactericidal quinolone followed by ofloxacin, ciprofloxacin and sparfloxacin in that order of potency. It can be seen from Table II that each strain showed similar sensitivity to the quinolones, despite having varying susceptibility to penicillin G.

The bactericidal profiles obtained for cefotaxime against the pneumococci demonstrate that a biphasic response did not occur and hence no OBC was obtained (Figure 2). The bactericidal activity of cefotaxime reduced with increasing bacterial resistance to penicillin G.

To compare the bactericidal activities of the quinolones

Table I. C_{max} values used to calculate BIs in this study

Drug	Dose (g)	C_{max} (mg/L)	Source of data (reference number)
Levofloxacin	0.5	5.21	19
Ofloxacin	0.4	5.85	20
Sparfloxacin	0.4	1.18	21
Ciprofloxacin	0.5	2.60	22
Cefotaxime	1.0	86.1	23

Table II. Bactericidal activity of four quinolones against four strains of *S. pneumoniae*[a] after 3 h at 37°C

	OBC mg/L (reduction in log cfu)			
Quinolone	C3LN4	269	16000	KPR
Levofloxacin	5 (2.2)	5 (2.4)	5 (2.0)	5 (2.6)
Ofloxacin	5 (2.1)	5 (2.0)	5 (1.9)	5 (2.3)
Sparfloxacin	3 (1.0)	3 (1.2)	5 (0.7)	3 (0.9)
Ciprofloxacin	3 (1.3)	3 (1.5)	5 (1.2)	3 (1.1)

[a] 269 and C3LN4 are penicillin G-sensitive; 16000 is penicillin G-intermediate; KPR is penicillin G-resistant. The results are an average of three determinations.

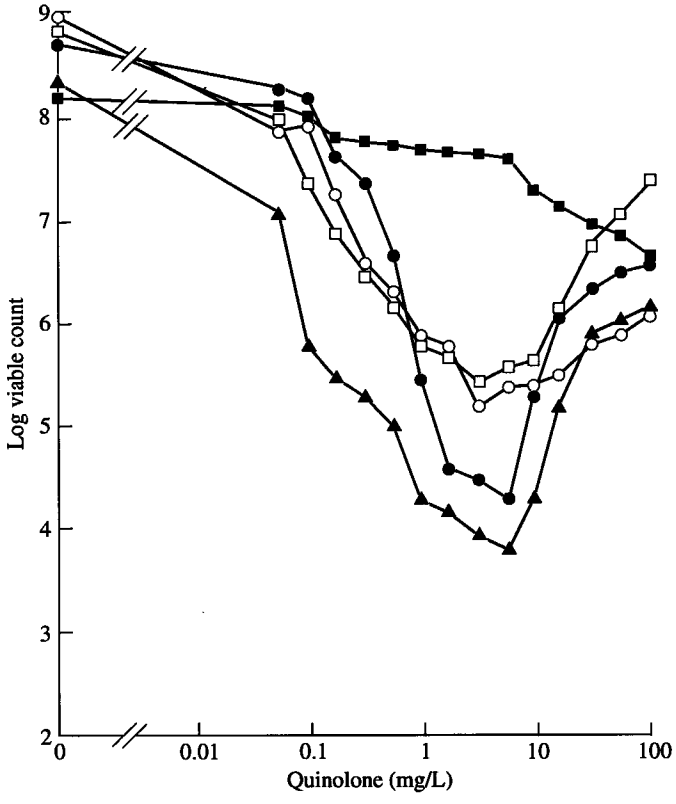

Figure 1. Quinolone bactericidal profiles against *S. pneumoniae* C3LN4. Ofloxacin (●), ciprofloxacin (○), levofloxacin (▲), sparfloxacin (□) and D-ofloxacin (■).

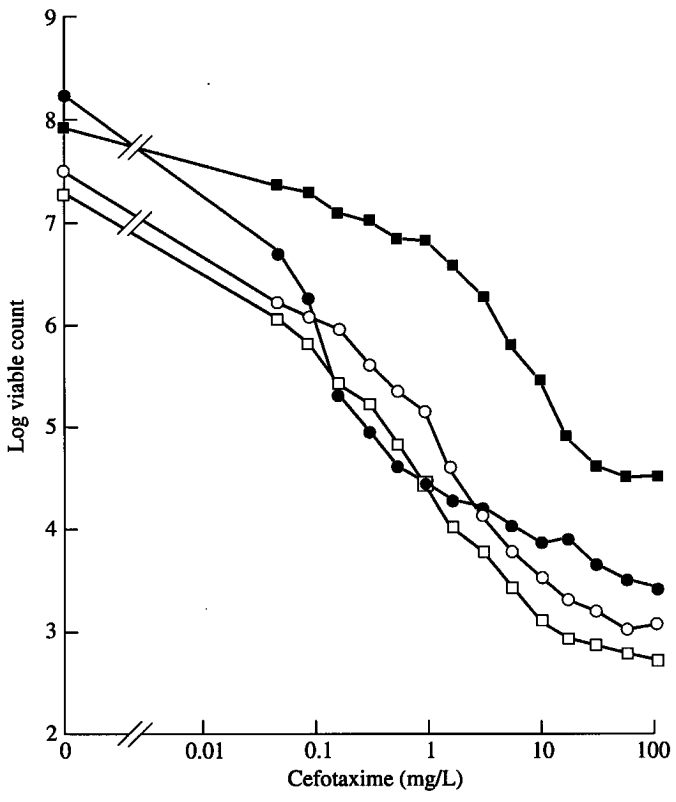

Figure 2. Cefotaxime bactericidal profiles against four strains of *S. pneumoniae*. Penicillin-resistant strain (■), penicillin-intermediate strain (●) and penicillin-sensitive strains C3LN4 (○) and 269 (□).

and cefotaxime against the pneumococci, the BIs were calculated (Figure 3). The bactericidal activity of D-ofloxacin was too poor to warrant calculation of a BI. The results confirmed levofloxacin as the most potent quinolone tested against all four pneumococci, exhibiting BIs on average 1.4 times greater than ofloxacin, despite ofloxacin having a slightly favourable C_{max} (Table I). Cefotaxime showed the highest overall BI against the penicillin-resistant pneumococci and against the penicillin-intermediate pneumococcus. However, against the penicillin-resistant strain, levofloxacin displayed the highest BI. Of the quinolones, ofloxacin was the second most potent against all four strains. Ciprofloxacin and sparfloxacin were considerably less bactericidal than ofloxacin. Sparfloxacin was the least bactericidal quinolone tested (except for D-ofloxacin). The relative potency of quinolones as measured by BI was very similar to that obtained using OBCs and log reduction in viability.

Discussion

The results of this study confirm the findings of previous in-vitro studies that show levofloxacin to be very active against *S. pneumoniae*[11,12] and confirm the results of studies using a lower respiratory tract model[15] and an artificial glass model.[16] Quinolone-susceptibility was found to be unrelated to penicillin-susceptibility, as was shown earlier by Visalli *et al.*[6] However, in this study, ofloxacin was more effective than sparfloxacin or ciprofloxacin, which is contrary to the findings of Spangler *et al.*[13] In addition, ciprofloxacin was slightly more potent than sparfloxacin, again contrary to the findings of Visalli *et al*.[6] This may be due to the varying methodologies and strains used. However, Spangler *et al.*[13] and Vissali *et al.*[6] did not account for drug pharmacokinetics. It is not likely, however, that the use of different strains would result in a large difference in the bactericidal activity of sparfloxacin, because in terms of log reduction in viability, all four strains in this study gave very similar results. Furthermore in a recent study by Barakett *et al.*,[17] the bactericidal activity of sparfloxacin against ten pneumococci was found to be comparable to our results. Unfortunately, Barakett and colleagues did not investigate the bactericidal activity of other quinolones.

The overall results obtained with quinolones using OBC data were very similar to those obtained using BI calculations, even though the C_{max} used for calculating the BI for sparfloxacin was 4.8 and 2.2 times lower than those used for ofloxacin or ciprofloxacin, respectively. This would suggest that previous studies comparing quinolones by their OBC are valid. However, if quinolones and other classes of

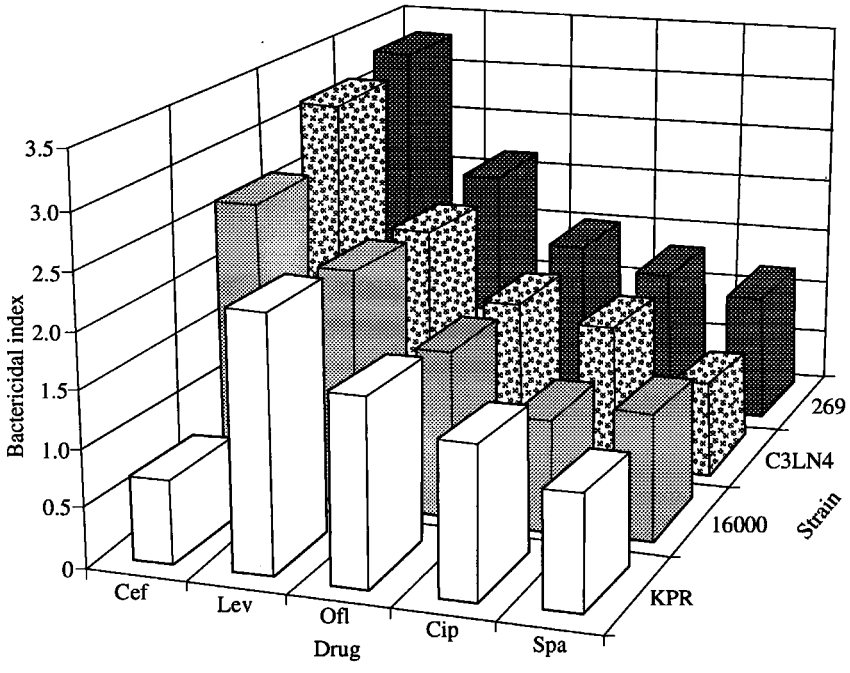

Figure 3. BIs for levofloxacin (Lev), cefotaxime (Cef), ofloxacin (Ofl), ciprofloxacin (Cip) and sparfloxacin (Spa) against four strains of *S. pneumoniae.*

antibacterial agent are to be compared, then calculation of BIs is a preferable method to use because other antibacterials agents such as cefotaxime do not show OBCs. Levofloxacin showed comparable BIs to cefotaxime, despite the peak serum level of levofloxacin being 17 times lower than that of cefotaxime. The other quinolones tested all showed lower BIs than cefotaxime and do not appear to be as effective as this drug. However, it is important to note that these findings would require clinical comparisons in order to investigate the accuracy of the BI method in predicting the bactericidal activity of these drugs *in vivo*.

Of those quinolones available in the near future, levofloxacin appears to be the most potent against *S. pneumoniae*, including both penicillin-sensitive and penicillin-resistant strains. However, in-vitro, developmental quinolones such as DU-6859a[14] and trovafloxacin[6,18] are even more potent against pneumococci. Further investigations are awaited.

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