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The effect of pharmacokinetics on the bactericidal activity of ciprofloxacin and sparfloxacin against *Streptococcus pneumoniae* and the emergence of resistance

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The pharmacokinetics of ciprofloxacin and sparfloxacin were simulated *in vitro* and the effects of pharmacodynamic parameters on bactericidal activity and the emergence of quinolone resistance were examined for *Streptococcus pneumoniae*. Simulated serum concentrations of ciprofloxacin 500 mg bd were more rapidly bactericidal than sparfloxacin 200 mg bd, despite lower values for the key pharmacodynamic parameters (AUC/MIC and C_{max}/MIC). This was possibly related to the slower oral absorption of sparfloxacin, which delayed achievement of the MIC compared with ciprofloxacin. In addition, sparfloxacin was shown to have similar bactericidal activity to ciprofloxacin when tested at the same concentrations, despite its four-fold better potency in MIC terms. The emergence of resistance following exposure to ciprofloxacin appeared to be dependent on the C_{max}/MIC ratio and the AUC above the MIC, but not the AUC/MIC ratio. Resistance (at least four-fold increase in MIC) developed when the C_{max}/MIC ratio was less than four or the AUC above the MIC was less than 10, and the resulting cultures regrew fully. In contrast, pneumococci with a two- to four-fold increase in sparfloxacin MIC were selected in the presence of serum concentrations of sparfloxacin despite a C_{max}/MIC ratio higher than 12, but these isolates remained clinically susceptible by breakpoint MIC and their growth was inhibited by repeated dosage of sparfloxacin. Nevertheless, the selection of pneumococci with reduced susceptibility, and the possibility of further mutation to highly resistant strains supports the use of quinolones that rapidly eradicate pneumococci at conventional doses and achieve concentrations, in both serum and tissues, which exceed at least 4 × MIC.

Introduction

Recent increases in the incidence of resistance to β-lactam and macrolide antibiotics amongst *Streptococcus pneumoniae*[1–3] have meant that quinolones are now being considered for use in the treatment of respiratory tract infections. In order to make this a viable and attractive option, the potencies of the fluoroquinolones against Gram-positive bacteria, particularly *S. pneumoniae*, have been improved. In addition, many of the new quinolones have extended serum half lives ($t_{½}$), which means that they can be dosed less frequently, often once daily, potentially increasing patient compliance.

The effect of these altered pharmacokinetic parameters and improved antibacterial potency (i.e. pharmacodynamics) on the efficacy of the new (third-generation) quinolones has been examined in a number of studies. The ratio of the area under the concentration in the serum–time curve over the first 24 h after dosing (AUC_{24}) to the MIC (AUC_{24}/MIC) is generally accepted to be the most predictive pharmacodynamic parameter,[4] with a value of >30–40 as the target AUC_{24}/MIC for optimal efficacy.[5,6] Studies have also been performed to examine the most predictive pharmacodynamic parameter for the selection of resistance to quinolones;[7–12] a few of these have looked at the selection of resistance in *S. pneumoniae*.[7–9] The parameter that has been suggested as most predictive for prevention of resistance selection is the ratio of the peak concentration of the quinolone in serum (C_{max}) to the MIC, with quoted target values for C_{max}/MIC ranging from 2.2 to 10.

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In the study described here, the effects on bactericidal activity of a prolonged $t_{½}$ and reduced C_{max} against *S. pneumoniae*, and the selection of quinolone resistance, were examined. Ciprofloxacin was used as the control quinolone and sparfloxacin was used as an example of a 'third-generation' quinolone with improved potency against *S. pneumoniae*. Sparfloxacin was chosen because it represents an extreme example with respect to pharmacokinetics, with the longest serum half-life of the new quinolones (15–22 h) and the lowest serum C_{max} (0.9 mg/L for the 200 mg dose).[13–15]

Materials and methods

Compounds

Ciprofloxacin (Bayer plc, Newbury, UK), sparfloxacin (Rhône-Poulenc Rorer, Eastbourne, UK) and trovafloxacin (Pfizer Laboratories, Sandwich, UK) were all used as soluble powders.

Bacterial strain

S. pneumoniae ATCC 6303 (ATCC, Manassass, VA, USA), an American Type Culture Collection control strain that is susceptible to all classes of antibiotic routinely tested against pneumococci, was used in these studies. The MIC of ciprofloxacin was 0.5 mg/L and of sparfloxacin was 0.125 mg/L for this strain, using the method described below.

MIC determinations

Serial two-fold dilutions of antibiotic were prepared in Mueller–Hinton agar (BBL Microbiology Systems, Cockeysville, MD, USA), supplemented with 5% (v/v) sterile defibrinated horse blood. The agar was inoculated with 10^5 cfu/spot of test organism and incubated for 18–24 h at 37°C. The MIC was determined as the lowest concentration of antibiotic to completely inhibit visible bacterial growth.

In vitro pharmacodynamic model

An open, one compartment, biexponential *in vitro* model was used.[16] The flow rate of the pump and the volumes in the flasks were set to simulate the elimination rate of the antibiotic with the shortest half-life (i.e. 4 h for ciprofloxacin), whereas sparfloxacin ($t_{½}$ = 16 h) concentrations were supplemented at regular intervals to simulate their slower elimination from man. The dilution rate of the bacterial cultures in the open system was therefore the same for all of the test antibiotics and the untreated control system. Repeated doses of antibiotic were administered automatically, using a computer-controlled pump. The medium used was Mueller–Hinton broth (Difco Laboratories, Detroit, MI, USA), supplemented with 5% (v/v) sterile, heat-treated horse serum. Samples were removed from the culture flask at regular time points for determination of the concentration of antibiotic and the number of viable bacteria present. Viable bacterial counts were determined by performing serial 10-fold dilutions of the samples and plating four dilutions in triplicate on to nutrient agar (Lab M, Bury, UK), supplemented with 5% (v/v) sterile horse blood. The numbers of colony-forming units (cfu) were determined following 24 h aerobic incubation at 37°C. Antibiotic concentrations were assayed microbiologically as below. Isolates from the samples taken at 24 h intervals were tested for quinolone susceptibility by agar dilution MIC determination (as described above).

Microbiological assays

Ciprofloxacin and sparfloxacin concentrations were assayed using a commercially available *Bacillus subtilis* NCTC 6633 spore suspension (Difco) grown in nutrient agar. Standard solutions were prepared in the appropriate dilution of Mueller–Hinton broth containing 5% horse serum. Samples were assayed in duplicate against standards over the concentration range of 5–0.16 mg/L (the limit of detection for the assay). The correlation coefficients for the regression lines of the standard solutions were not less than 0.991 and 80% were $\geq$0.996.

Antibiotic doses simulated

The doses of ciprofloxacin and sparfloxacin simulated in the *in vitro* pharmacodynamic model and their relevant pharmacokinetic parameters are summarized in Table I. The doses chosen were those recommended by the manufacturers for the treatment of respiratory tract infection in the community. In order to determine the effects of differing pharmacokinetic parameters, however, ciprofloxacin was also tested using the pharmacokinetic parameters of sparfloxacin.

Results

Bactericidal activity

The 500 mg ciprofloxacin and 200 mg sparfloxacin doses simulated in the *in vitro* model give C_{max}/MIC ratios of 4.74 and 7.2 and AUC/MIC ratios of 38 and 275, respectively, when dosed twice daily for 24 h (Table I). Despite the higher values for sparfloxacin for these two key pharmacodynamic parameters, simulated serum concentrations of ciprofloxacin were reproducibly more bactericidal than those of sparfloxacin against *S. pneumoniae* ATCC 6303 over the first 8 h after dosing (Figure 1). Also shown in Figure 1 are the mean concentrations of ciprofloxacin and sparfloxacin achieved in these studies. Ciprofloxacin is absorbed rapidly following oral administration in humans,

Table I. Pharmacokinetic parameters for the doses of ciprofloxacin and sparfloxacin simulated in the *in vitro* pharmacodymanic model

Compound	Dose, regimen (reference)	Body site	Pharmacokinetic parameter						
			C_{max} (mg/L)	T_{max} (h)	$t_{½}$ (h)	$AUC_{0–24}$ (mg·h/L)	C_{max}/MIC ratio	AUC/MIC ratio (h)	AUC > MIC (mg·h/L)
Ciprofloxacin	500 mg bd (17)	serum	2.37	1.11	4.5	19.2	4.74	38.4	10.54
	spa. PK bd (14)	serum	0.9	5.33	15.2	34.4	1.80	68.8	3.45
	750 mg bd (17)	serum	2.96	1.56	5.3	31.2	5.92	62.4	17.96
	500 mg bd (27)	blister fluid	1.2	2.6	5.6	23.2	2.4	46.4	5.65
	750 mg bd (27)	blister fluid	1.8	2.6	5.6	34.8	3.6	69.6	8.43
Sparfloxacin	200 mg bd (14)	serum	0.9	5.33	15.2	34.4	7.2	275.2	11.10
	400 mg od (15)	serum	1.6	2.7	17.6	32.3	12.8	258.4	10.52

reaching a C_{max} between 1 and 1.5 h after dosing and achieving the MIC for *S. pneumoniae* ATCC 6303 (0.5 mg/L) *c.* 10 min after dosing. Sparfloxacin, on the other hand, is absorbed more slowly, reaching a C_{max} *c.* 5 h after dosing and achieving the MIC for *S. pneumoniae* ATCC 6303 (0.125 mg/L) *c.* 1 h after dosing. It appears that sparfloxacin does not produce a rapid bactericidal effect against *S. pneumoniae* ATCC 6303 until the concentration exceeds the MIC, i.e. between the 1 h and 2 h samples (Figure 1). In contrast, ciprofloxacin was rapidly bactericidal within the first hour after dosing and the overall bactericidal effect was greater than that seen with sparfloxacin for at least 8 h. After 24 h of twice daily dosing, the antibacterial efficacy of the two agents was similar, however, with *c.* 2 $\log_{10}$ cfu/mL remaining (Figure 2); by 48 h, no viable bacteria were detectable in either culture ($<$1.22 $\log_{10}$ cfu/mL). The culture treated with ciprofloxacin also contained $<$1.22 $\log_{10}$ cfu/mL after 72 h of dosing, whereas the culture treated with sparfloxacin contained 2 $\log_{10}$ cfu/mL at this time.

In order to examine whether the differences in bactericidal activity seen over the first 8 h were owing to differences in pharmacokinetics or differences in mechanism of action, ciprofloxacin was tested using sparfloxacin pharmacokinetic parameters. In addition, a higher dose of sparfloxacin (400 mg) was tested (Figure 3). The initial rate of bacterial kill in the culture treated with ciprofloxacin tested using sparfloxacin pharmacokinetics was equivalent to that of sparfloxacin, and slower than that of ciprofloxacin tested using ciprofloxacin pharmacokinetic parameters. The concentration of antibiotic present at the extrapolated onset of rapid bactericidal activity (90 min) was *c.* 0.2 mg/L in the cultures exposed to sparfloxacin pharmacokinetics. Although this was almost twice the MIC of sparfloxacin, it was only approximately half of the MIC of ciprofloxacin for *S. pneumoniae* ATCC 6303.

Sparfloxacin was also tested at concentrations simulating a higher dose (400 mg) and the rate of bacterial kill paralleled that seen with the lower dose of sparfloxacin (200 mg), but the bacterial growth before the onset of bactericidal activity was reduced by *c.* 0.5 $\log_{10}$.

This study was also continued up to 72 h and, as seen in the previous study, the culture treated with ciprofloxacin concentrations simulating those achieved in serum following oral dosage of 500 mg bd contained $<$1.22 $\log_{10}$ cfu/mL at 72 h (Table II), whereas the culture treated with sparfloxacin 200 mg bd contained 2 $\log_{10}$ cfu/mL at this time. The culture treated with 400 mg sparfloxacin od for the first day and 200 mg od for the following two days contained 2.22 $\log_{10}$ cfu/mL at 72 h, such that the overall effect was similar to that seen with the 200 mg bd regimen (Table II). The culture treated with ciprofloxacin, but using sparfloxacin pharmacokinetics, had fully regrown (to 7.93 $\log_{10}$ cfu/mL) by 48 h after the start of dosing. The reason for this regrowth after the initial good bactericidal activity up to 24 h (Table II) is described below.

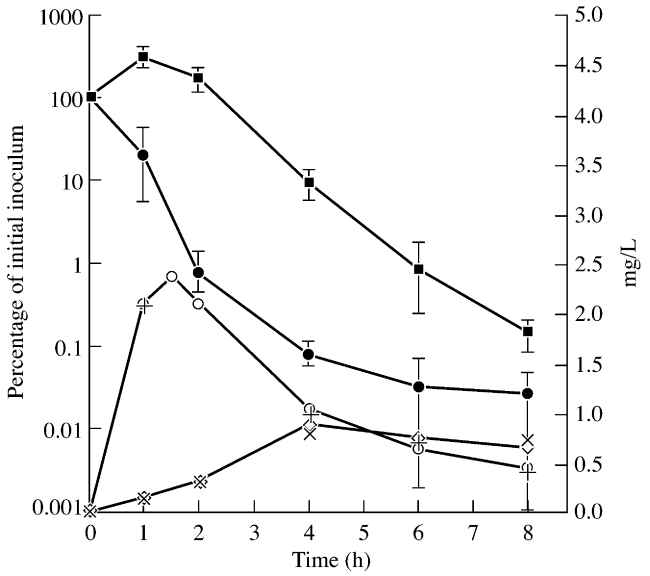

Figure 1. Mean concentrations of ciprofloxacin (○, +) and sparfloxacin (◇, ×) achieved (in the serum of man[14,17] and the *in vitro* model) following oral dosage of 500 mg bd and 200 mg bd, respectively, and the bactericidal activities of these concentrations of ciprofloxacin (●) and sparfloxacin (■) against *S. pneumoniae* ATCC 6303. Error bars represent the range of three studies.

Emergence of quinolone resistance

In the first 3 day study described above (Figure 2), the culture treated with ciprofloxacin concentrations simulating a 500 mg bd regimen contained no detectable organisms between 26 and 72 h after the start of dosing. However, *S. pneumoniae* (2 $\log_{10}$ cfu/mL) were isolated at 72 h from the culture treated with sparfloxacin 200 mg bd. When tested for antibiotic susceptibility, the *S. pneumoniae* isolated 72 h after the start of treatment with sparfloxacin were four-fold less susceptible to both ciprofloxacin and sparfloxacin than the parent culture.

In the second study, viable streptococci were reduced to below or around the limit of detection by 24 h after dosing in all treated cultures (Table II), but in those treated with either regimen of sparfloxacin, low numbers of bacteria (2–4 $\log_{10}$ cfu/mL) were detected at 48 and 72 h after dosing. When the quinolone susceptibilities of these cultures were tested, it was found that they were four- to eight-fold less susceptible to ciprofloxacin and trovafloxacin and two- to four-fold less susceptible to sparfloxacin than the parent culture (Table II).

In addition, the culture treated with ciprofloxacin using sparfloxacin 200 mg bd pharmacokinetic parameters was four-fold less susceptible to ciprofloxacin and trovafloxacin and two-fold less susceptible to sparfloxacin after 48 h (Table II). The MIC of ciprofloxacin for this culture had increased to 2 mg/L, which was above the C_{max} used in this model (sparfloxacin pharmacokinetics, C_{max} of 0.9 mg/L) and resulted in the culture regrowing fully by 48 h. Ciprofloxacin 500 mg bd, on the other hand, reduced the numbers of viable bacteria below the limit of detection by 24 h and no viable organisms were detected up to the end of the study (72 h).

The full regrowth, or 'bacteriological failure', seen with the lower C_{max} of ciprofloxacin was confirmed by simulating concentrations of ciprofloxacin achieved in the blister fluid of humans following oral administration of 500 mg bd and 750 mg bd (Figure 4). Concentrations of ciprofloxacin achieved in blister fluid have a delayed and reduced C_{max} compared with that achieved in serum. In both cultures where blister fluid concentrations were simulated, the pneumococci regrew by 24 h after the start of dosing and the organisms isolated were four-fold less susceptible to ciprofloxacin than the parent culture at that time (Figure 4). In contrast, the numbers of viable streptococci in the cultures exposed to simulated serum concentrations of ciprofloxacin fell below 2 $\log_{10}$ cfu/mL by 28 h after the start of dosing and no emergence of resistance was seen in these cultures.

Discussion

In these studies, ciprofloxacin 500 mg bd was reproducibly more bactericidal over the first 8 h after dosing than sparfloxacin 200 mg bd against *S. pneumoniae* ATCC 6303, despite sparfloxacin having higher values for the two key pharmacodynamic parameters considered important for optimum bacteriological efficacy of quinolones (AUC/MIC and C_{max}/MIC). When the pharmacodynamics of the two compounds were examined more closely, however, it was clear that the slower absorption of sparfloxacin following oral administration meant that the MIC for *S. pneumoniae* ATCC 6303 was achieved much later than with ciprofloxacin. Since the rate of bactericidal activity of quinolones is concentration dependent, it was logical that

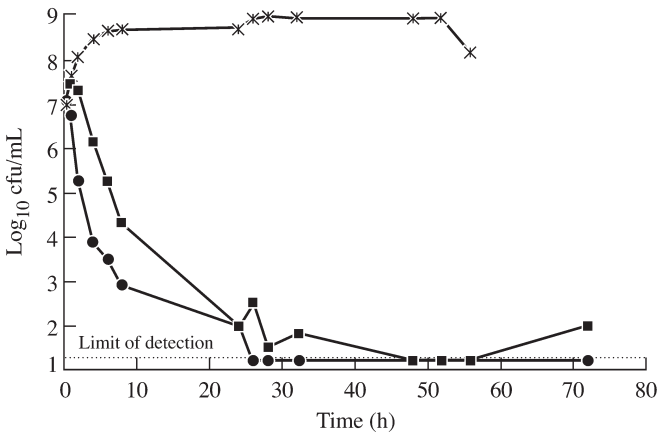

Figure 2. Bactericidal activities of simulated serum concentrations achieved following oral dosage of ciprofloxacin 500 mg bd (●) and sparfloxacin 200 mg bd (■) compared with an untreated control culture (×) of *S. pneumoniae* ATCC 6303.

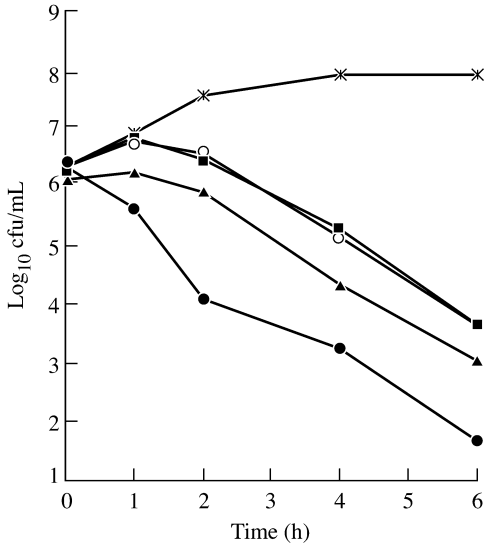

Figure 3. Bactericidal activities of simulated concentrations achieved in the serum of man following oral dosage of 500 mg ciprofloxacin (●), ciprofloxacin using sparfloxacin 200 mg pharmacokinetics (○), 200 mg sparfloxacin (■) and 400 mg sparfloxacin (▲) compared with an untreated control culture (×) of *S. pneumoniae* ATCC 6303.

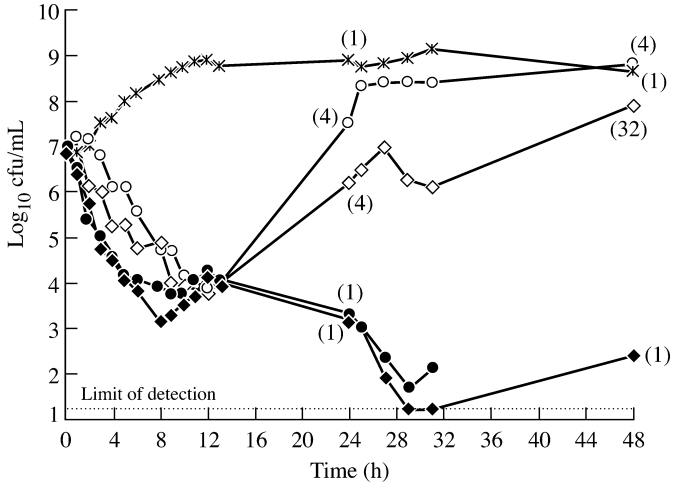

Figure 4. Bactericidal activities of simulated concentrations achieved in the serum (filled symbols) and blister fluid (open symbols) of man following oral dosage of ciprofloxacin 500 mg bd (●, ○) and 750 mg bd (◆, ◇) compared with an untreated control culture (×) of *S. pneumoniae* ATCC 6303. Figures in parentheses represent fold increases in *S. pneumoniae* MICs compared with 0 h.

ciprofloxacin should be more bactericidal initially. Moreover, these data were consistent with *in vivo* data from a murine pneumonia model in which ciprofloxacin attained higher maximal bactericidal effect values, a steeper killing slope and a shorter time to maximal bactericidal effect in comparison with sparfloxacin for the highest doses tested.[18]

These data also show that the bactericidal activities of ciprofloxacin and sparfloxacin were similar when they were tested at the same concentrations (sparfloxacin pharmacokinetics), despite the four-fold greater potency of sparfloxacin against *S. pneumoniae* ATCC 6303 in MIC terms. This result was consistent with studies by George & Morrissey,[19] in which sparfloxacin and ciprofloxacin had equivalent bactericidal activity when tested at the same concentrations (up to 10 mg/L) against *S. pneumoniae*. The equivalent bactericidal activity but different MICs for these two quinolones could be due to differences in their predominant target site in pneumococci. Ciprofloxacin has been shown to primarily target topoisomerase IV in *S. pneumoniae*,[20] whereas genetic studies have indicated that sparfloxacin predominantly inhibits DNA gyrase.[21,22] Cell-free data, however, show ciprofloxacin and sparfloxacin to have identical IC_{50}s for topoisomerase IV from *S. pneumoniae*, and a higher affinity for topoisomerase IV than for DNA gyrase.[23,24] In addition, ciprofloxacin and sparfloxacin had similar IC_{50}s for pneumococcal DNA gyrase.[23,24] The reason for the discrepancy between genetic and enzyme inhibition studies with *S. pneumoniae* has not yet been elucidated. This is different from the situation in *Staphylococcus aureus*, where topoisomerase IV is the predominant target for both ciprofloxacin and sparfloxacin.[25] Unpublished data in the *in vitro* pharmacodynamic model (C. E. Thorburn) show that sparfloxacin was more rapidly bactericidal than ciprofloxacin against *S. aureus* when both were tested using sparfloxacin pharmacokinetics.

As well as being less rapidly bactericidal than ciprofloxacin against *S. pneumoniae* ATCC 6303, exposure to simulated sparfloxacin 200 mg bd serum concentrations reproducibly selected variants with reduced quinolone susceptibility, whereas this was not the case following exposure to ciprofloxacin 500 mg bd serum concentrations. This may be related to the slower bactericidal activity of sparfloxacin, giving the bacteria more chance to divide and mutate before they are killed. In addition, a higher mutation rate to decreased susceptibility has previously been reported for *S. pneumoniae* exposed to sparfloxacin (1 in 5.6 $\log_{10}$) compared with ciprofloxacin (1 in 7 $\log_{10}$).[26] Resistant variants were selected following exposure to ciprofloxacin, however, when it was tested using a lower C_{max}, slower absorption rate and longer half-life (i.e. sparfloxacin pharmacokinetics). This raised the question as to whether resistant isolates would be selected following exposure to concentrations of ciprofloxacin achieved in interstitial fluid which, although having the same AUC as serum concentrations, have a lower C_{max} and slower absorption rate, which contributes to a slightly longer $t_{½}$. Blister fluid concentrations of antibiotics are considered to be representative of those achieved in some tissues and have been measured for ciprofloxacin by Crump *et al.*[27] These concentrations were simulated in the *in vitro* model for two dose levels of ciprofloxacin and compared with serum concentrations achieved in humans for the same two

Table II. Bactericidal activity and changes in susceptibility following exposure of *S. pneumoniae* ATCC 6303 to simulated serum concentrations of ciprofloxacin and sparfloxacin for 3 days

Compound, dose and regimen	Time (h)	Viable bacteria count (log_{10} cfu/mL)	MIC (mg/L)		
			CIP	SPX	TVA
All	0	6.11–6.40	0.5	0.12	0.06
Untreated control	24	8.79	0.5	0.12	0.06
	72	8.52	0.5	0.12	0.06
Ciprofloxacin, 500 mg bd	24	⩽1.22	–	–	–
	48	⩽1.22	–	–	–
	72	⩽1.22	–	–	–
Ciprofloxacin, 200 mg bd (sparfloxacin pharmacokinetics)	24	1.52	0.5	0.12	0.06
	48	7.93	**2**	0.25	**0.25**
Sparfloxacin, 200 mg bd	24	⩽1.22	–	–	–
	48	3.22	**2**	0.25	**0.25**
	72	2.07	**4**	**0.5**	**0.5**
Sparfloxacin, 400 mg od then 200 mg od	24	⩽1.22	–	–	–
	48	4.12	**2**	0.25	**0.25**
	72	2.22	**2**	0.25	**0.5**

CIP, ciprofloxacin; SPX, sparfloxacin; TVA, trovafloxacin.
Bold type indicates a ⩾4-fold increase in MIC compared with the parent culture.

doses. Exposure to simulated blister fluid concentrations of both doses resulted in the selection of resistant variants of *S. pneumoniae*, whereas exposure to simulated serum concentrations did not select resistant variants.

When an attempt was made to correlate a pharmacodynamic parameter with the potential of ciprofloxacin to select resistant pneumococci, the AUC_{24}/MIC ratio was examined initially. This value was similar, however, for concentrations achieved in serum and blister fluid at each dose level (*c.* 40 for the 500 mg dose and 60–70 for the 750 mg dose). The only pharmacodynamic parameters that were higher for serum concentrations of ciprofloxacin than for blister fluid concentrations were the C_{max} values (and consequently the C_{max}/MIC ratios) and the AUC above the MIC. The C_{max}/MIC ratios were >4 for serum concentrations of ciprofloxacin, and <4 for blister fluid concentrations, with resistance selected when the C_{max}/MIC ratio was 3.6, but not when it was 4.7. This value is consistent with published data on the target C_{max}/MIC ratio for ciprofloxacin to prevent the selection of resistance in Gram-positive pathogens. In studies against *S. aureus*[12] and *S. pneumoniae*,[7–9] the target C_{max}/MIC ratios recommended to prevent the selection of resistance ranged from 2.2 to 5. Higher target values for the C_{max}/MIC ratio (8–10) have been reported by workers using Gram-negative pathogens, such as *Klebsiella pneumoniae*[10] and *Pseudomonas aeruginosa*.[10–12] This suggests that the target C_{max}/MIC ratio necessary to prevent the selection of quinolone resistance may be species specific.

The AUC above the MIC (AUC > MIC) was also found to correlate with the selection of resistant *S. pneumoniae* following exposure to ciprofloxacin, with resistance selected at AUC > MIC values ⩽8.4 but not when the AUC > MIC was ⩾10.5. Few other workers have examined the relationship between AUC > MIC of quinolones and selection of resistance, but our results are consistent with those of Marchbanks *et al.*,[12] who reported that resistant *S. aureus* were selected following exposure to ciprofloxacin concentrations with an AUC > MIC of 4.7, but not when the AUC > MIC was 35.4.

Exposure to sparfloxacin, on the other hand, yielded less susceptible variants of *S. pneumoniae* in the presence of C_{max}/MIC ratios >12 and AUC > MIC values >11. Sparfloxacin may not follow the same rules as ciprofloxacin, however, because of a different mechanism of action.

The resistant isolates selected following exposure to ciprofloxacin and sparfloxacin were generally four- to eight-fold less susceptible to ciprofloxacin and trovafloxacin, and two- to four-fold less susceptible to sparfloxacin than the parent culture. Susceptibility determinations were

performed for the quinolones with and without 20 mg/L reserpine, and there was no difference in the MICs obtained. This suggests that efflux did not play a role in the resistance of these isolates. The greater effect on the potency of ciprofloxacin and trovafloxacin compared with sparfloxacin suggests that these variants contain mutations in *grlA* or *grlB*, the genes encoding the ParC and ParE subunits of topoisomerase IV, rather than *gyrA* or *gyrB*, which encode the GyrA and GyrB subunits of DNA gyrase. For ciprofloxacin, this is consistent with the findings of Pan *et al.*,[20] who reported that ParC mutations preceded those in GyrA in both laboratory strains and clinical isolates, and Gootz *et al.*,[28] who showed that first-step mutants of pneumococci with ciprofloxacin MICs of 4–8 mg/L and trovafloxacin MICs of 0.25–0.5 mg/L contained changes in the serine at position 80 of *grlA*. However, it is not consistent with published genetic studies on sparfloxacin-selected variants, which were found to contain only mutations in GyrA.[21,22] The low level of sparfloxacin resistance selected also meant that these variants of *S. pneumoniae* ATCC 6303 were still clinically susceptible to sparfloxacin according to the breakpoint MIC (0.5 mg/L). In accordance, these cultures contained at least 3 $\log_{10}$ fewer organisms by the end of the study than were present at the start of dosing, i.e. bacteriological efficacy of sparfloxacin was seen, despite the selection of isolates with reduced susceptibility.

Overall, these data show that the initial bactericidal activity observed following oral administration of a quinolone, and its potential to select resistance, are dependent on pharmacodynamic parameters. The main pharmacodynamic parameter corresponding with a rapid bactericidal effect was a high, early C_{max}. A C_{max}/MIC ratio >4 and an AUC > MIC value >10 corresponded to a lower potential to select resistance for ciprofloxacin. This suggests that ciprofloxacin-resistant pneumococci are likely to be selected at body sites where low C_{max} values are achieved or from strains of pneumococci which are less susceptible before treatment (i.e. ciprofloxacin MICs > 0.5 mg/L). Whether this target C_{max}/MIC ratio is predictive for the emergence of resistant pneumococci following exposure to other quinolones with the same target site as ciprofloxacin remains to be seen. For sparfloxacin, less susceptible variants of *S. pneumoniae* were isolated, even when C_{max}/MIC ratios were as high as 12. These isolates were not clinically resistant to sparfloxacin, but could represent a first-step mutation, which may give rise to further mutations and a higher level of resistance. It therefore appears that a C_{max}/MIC ratio >4, achieved soon after dosing, should maximize bactericidal effect and minimize the potential for the selection of quinolone-resistant pneumococci.

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