Interactions of the 4-quinolones with other antibacterials

C. S. LEWIN* and J. T. SMITH†

Microbiology Section, Department of Pharmaceutics, The School of Pharmacy, London University, Brunswick Square, London WC1N 1AX

Summary. The effect of sub-inhibitory concentrations of 16 antibacterials on the bactericidal activity of the 4-quinolones nalidixic acid, ciprofloxacin and ofloxacin against *Escherichia coli* KL16 in nutrient broth was investigated. Sub-inhibitory concentrations of rifampicin, clindamycin, chloramphenicol, erythromycin or tetracycline antagonised the bactericidal activity of the 4-quinolones. Conversely, all seven aminoglycosides tested enhanced the bactericidal activity of the 4-quinolones whereas the cell wall antagonists, azlocillin, mezlocillin, ceftazidime and vancomycin had no effect on the bactericidal activity of the 4-quinolones.

Introduction

MIC (minimum inhibitory concentration) studies suggest that little interaction occurs between the 4-quinolones and other antibacterials (Neu and Labthavikul, 1982; Van der Auwera, 1985; Davies and Cohen, 1985; Wolfson and Hooper, 1985; Smith *et al.*, 1986). However, MICs provide information only about the inhibition of bacterial multiplication and provide no information on the bacterial killing process (Smith, 1984) which may be important in neutropenic patients. As protein and RNA synthesis are known to be prerequisites for the full bactericidal activity of the 4-quinolones (Dietz *et al.*, 1966; Smith, 1984), it is not surprising that the protein synthesis inhibitor chloramphenicol and the RNA synthesis inhibitor rifampicin have been found to antagonise the bactericidal activity of the 4-quinolones (Smith, 1984) although they do not affect the bacteriostatic activity of the 4-quinolones. The effects of sub-inhibitory concentrations of 16 different antibacterials on the bactericidal activity of nalidixic acid, ciprofloxacin and ofloxacin against *Escherichia coli* KL16 in nutrient broth were therefore investigated.

Materials and methods

Antibacterials

The following antibacterial agents were obtained from the indicated suppliers: amikacin (Bristol Laboratories), azlocillin (Bayer), ceftazidime (Glaxo), chloramphenicol

(Parke-Davis), ciprofloxacin (Bayer), clindamycin (Upjohn), erythromycin (Eli Lilly and Co.), gentamicin (Roussel Laboratories), kanamycin (Bristol Laboratories), mezlocillin (Bayer), nalidixic acid (Sterling-Winthrop), netilmicin (Schering Corporation), ofloxacin (Hoeschst), rifampicin (Merrell Dow Pharmaceuticals), sisomicin (Schering Corporation), streptomycin (Evans Medical Ltd), tetracycline (Lederle Laboratories), tobramycin and vancomycin (Eli Lilly and Co.).

All except erythromycin, nalidixic acid, ofloxacin and rifampicin were dissolved in sterile distilled water. Rifampicin and erythromycin were dissolved in absolute alcohol or dimethyl sulphoxide. Nalidixic acid and ofloxacin were first dissolved in 0.5 M NaOH (0.02 ml/mg) before being diluted to appropriate concentrations in sterile distilled water.

Determination of bactericidal activity

The 4-quinolones cause filamentation of bacteria, which increases the turbidity of liquid cultures, so bacterial killing by the 4-quinolones cannot be judged by light scattering or optical density measurements (Smith, 1984). The bactericidal activity of the 4-quinolones studied was measured therefore by determining viable counts.

Nutrient Broth No. 2 (Oxoid) was inoculated with *E. coli* KL16 and incubated overnight at 37°C.

Sterile double strength nutrient broth (5 ml) was added to the aqueous drug (or drugs) and the volume was made up to 9.8 ml with sterile distilled water. The antimicrobial solutions were incubated at 37°C for at least 15 min and then 0.2 ml of an overnight culture of *E. coli* KL16 which contained *c.* 5×10^8 cfu/ml was added at time zero to complete the reaction mixtures. The viable count at time zero was *c.* 10^7 cfu/ml. At 30-min intervals, 0.1-ml samples from each reaction mixture were taken and serial 10-fold dilution were made in sterile nutrient broth. A 0.1-ml sample of each dilution was plated on to nutrient

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* Present address: Bacteriology Department, Medical School, Teviot Place, Edinburgh EH8 9AG.

† Correspondence should be sent to Professor J. T. Smith.

agar and the number of colonies was counted after incubation overnight at 37°C. The results obtained were used to calculate the 99% kill time against *E. coli* KL16 of the 4-quinolone with and without a sub-inhibitory concentration of a second antibacterial.

Results

Bacteriostatic concentrations of rifampicin or

chloramphenicol reduced the bactericidal activity of ciprofloxacin and totally inhibited the bactericidal activity of nalidixic acid against *E. coli* KL16 in nutrient broth. When sub-inhibitory concentrations of these two drugs were combined with nalidixic acid 50 mg/L or ciprofloxacin 0.015 mg/L the time taken to kill 99% of the *E. coli* KL16 in nutrient broth was greater than when the 4-quinolones were used alone (tables I and II).

Table I. Interactions between nalidixic acid 50 mg/L and other antibacterials against *E. coli* KL16

Non-quinolone antibacterials and sub-inhibitory concentrations (mg/L)	MIC (mg/L)	99% kill times (min)* with nalidixic acid		
		4-quinolone alone	4-quinolone + other drug	Change (%)
Streptomycin (2)	3	153	130	-23
Gentamicin (0.2)	0.3	163	116	-47
Kanamycin (0.8)	1	156	106	-50
Tobramycin (0.4)	0.5	191	98	-93
Amikacin (0.4)	0.5	168	127	-41
Sisomicin (0.4)	0.5	127	72	-55
Netilmicin (0.4)	0.5	142	86	-56
Rifampicin (10)	15	128	252	+124
Tetracycline (0.3)	2	142	260	+118
Chloramphenicol (1)	4	141	175	+34
Erythromycin (30)	40	178	> 300	> +122
Clindamycin (10)	30	172	> 240	> +68
Vancomycin (0.4)	3	154	154	0
Azlocillin (1)	7.5	187	187	0
Mezlocillin (0.3)	1.5	183	183	0
Ceftazidime (0.03)	0.15	201	201	0

* See *Methods*.

Table II. Interactions between ciprofloxacin 0.015 mg/L and other antibacterials against *E. coli* KL16

Non-quinolone antibacterials and sub-inhibitory concentrations (mg/L)	MIC (mg/L)	99% kill times (min) with ciprofloxacin		
		4-quinolone alone	4-quinolone + other drug	Change (%)
Streptomycin (2)	3	168	128	-40
Gentamicin (0.2)	0.3	156	128	-28
Kanamycin (0.8)	1	137	108	-29
Tobramycin (0.4)	0.5	194	141	-53
Amikacin (0.4)	0.5	117	106	-11
Sisomicin (0.4)	0.5	166	85	-81
Netilmicin (0.4)	0.5	160	99	-61
Rifampicin (10)	15	143	146	+3
Tetracycline (0.3)	2	138	241	+103
Chloramphenicol (1)	4	128	193	+65
Erythromycin (30)	40	180	> 300	> +120
Clindamycin (10)	30	200	> 240	> +40
Vancomycin (0.4)	3	154	154	0
Azlocillin (1)	7.5	185	185	0
Mezlocillin (0.3)	1.5	210	210	0
Ceftazidime (0.03)	0.15	210	210	0

Sub-inhibitory concentrations of three other inhibitors of protein synthesis, tetracycline 0.03 mg/L, erythromycin 30 mg/L and clindamycin 10 mg/L were also tested for their effect on the bactericidal activity of the 4-quinolones. All three antibacterials increased the 99% kill times of nalidixic acid 50 mg/L (fig. 1 and table I) and of ciprofloxacin 0.015 mg/L (fig. 1 and table II) against *E. coli* KL16 in nutrient broth. The bactericidal activity of the two 4-quinolones appears, therefore,

to be antagonised by these inhibitors of protein synthesis at sub-inhibitory concentrations.

The aminoglycosides were similarly examined. These antimicrobials also inhibit bacterial protein synthesis and antagonism of the bactericidal activity of the 4-quinolones might have been expected. However, the bactericidal activities of nalidixic acid 50 mg/L (fig. 2 and table I) and of ciprofloxacin 0.015 mg/L (fig. 2 and table II) were enhanced by a sub-inhibitory concentration of the L-aminoglyco-

Fig. 1. Survival of *E. coli* KL16 in nutrient broth with antibacterials. A 1 in 50 dilution of an overnight culture was made in nutrient broth containing nalidixic acid 50 mg/L (a, ○—○), ciprofloxacin 0.015 mg/L (b, ○—○), tetracycline 0.3 mg/L (a and b, ●—●), nalidixic acid + tetracycline (a, □—□) or ciprofloxacin + tetracycline (b, □—□). Samples were taken at 30-min intervals and bacterial survival was estimated by viable counts on nutrient agar.

Fig. 2. Survival of *E. coli* KL16 in nutrient broth containing nalidixic acid 50 mg/L (a, ○—○), ciprofloxacin 0.015 mg/L (b, ○—○), streptomycin 2 mg/L (a and b, ●—●), nalidixic acid + streptomycin (a, □—□) or ciprofloxacin + streptomycin (b, □—□).

Table III. Interactions between ofloxacin 0.06 mg/L and other antibacterials against *E. coli* KL16

Non-quinolone antibacterials and sub-inhibitory concentrations (mg/L)	MIC (mg/L)	99% kill times (min) with ofloxacin		
		4-quinolone alone	4-quinolone + other drug	Change (%)
Streptomycin (2)	3	161	133	-28
Gentamicin (0.2)	0.3	161	141	-20
Kanamycin (0.8)	1	170	154	-16
Tobramycin (0.4)	0.5	170	127	-43
Amikacin (0.4)	0.5	165	138	-27
Sisomicin (0.4)	0.5	132	118	-14
Netilmicin (0.4)	0.5	165	121	-44
Rifampicin (10)	15	132	153	+21
Tetracycline (0.3)	2	133	> 240	> +107
Chloramphenicol (1)	4	133	236	103
Erythromycin (30)	40	148	> 240	> +92
Clindamycin (10)	30	148	187	+39
Vancomycin (0.4)	3	156	155	-1
Azlocillin (1)	7.5	156	158	+2
Mezlocillin (0.3)	1.5	152	150	-2
Ceftazidime (0.03)	0.15	152	150	-2

side streptomycin (2 mg/L). The effect was progressive with the bactericidal activity of nalidixic acid 50 mg/L being increasingly enhanced by streptomycin 2 mg/L over 7 h (fig. 3).

The bactericidal activities of nalidixic acid

Fig. 3. Survival of *E. coli* KL16 in nutrient broth containing nalidixic acid 50 mg/L (O—O), streptomycin 2 mg/L (●—●) or nalidixic acid + streptomycin (■—■).

50 mg/L (fig. 4 and table I) and of ciprofloxacin 0.015 mg/L (fig. 4 and table II) were also enhanced by a sub-inhibitory concentration of the D-aminoglycoside tobramycin (0.4 mg/L). When a further five aminoglycosides were tested they also enhanced the bactericidal activity of nalidixic acid 50 mg/L (table I) and of ciprofloxacin 0.015 mg/L (table II) against *E. coli* KL16 in nutrient broth. Therefore, the aminoglycosides appear to enhance the bactericidal activity of ciprofloxacin and nalidixic acid, whereas the other protein synthesis inhibitors antagonise the 4-quinolone bactericidal activity.

A beneficial interaction often occurs between two antibacterials when the drug concentrations are sub-optimal rather than optimal. However, sisomicin 0.4 mg/L was not only able to enhance the bactericidal activity of ciprofloxacin 0.015 mg/L against *E. coli* KL16 but when ciprofloxacin was used at its maximum bactericidal concentration of 0.15 mg/L, potentiation still occurred (fig. 5). Hence, even the optimal bactericidal activity of the 4-quinolones was enhanced by the aminoglycosides.

The interactions of the two 4-quinolones with sub-inhibitory concentrations of inhibitors of cell-wall synthesis, including vancomycin which has already been used in combination with ciprofloxacin (Smith *et al.*, 1988), were also examined. Vancomycin at a sub-inhibitory concentration of 0.4 mg/L had no effect on the bactericidal activity of either nalidixic acid 50 mg/L or of ciprofloxacin 0.015 mg/L against *E. coli* KL16 in nutrient broth (fig. 6 and tables I and II). The bactericidal

Fig. 4. Survival of *E. coli* KL16 in nutrient broth containing nalidixic acid 50 mg/L (a, ○—○), ciprofloxacin 0.015 mg/L (b, ○—○), tobramycin 0.4 mg/L (a and b, ●—●), nalidixic acid + tobramycin (a, □—□) or ciprofloxacin + tobramycin (b, □—□).

Fig. 5. Survival of *E. coli* KL16 in nutrient broth containing ciprofloxacin 0.015 mg/L (a, ○—○) or 0.15 mg/L (b, ○—○), sisomicin 0.4 mg/L (a and b, ●—●), ciprofloxacin 0.015 mg/L + sisomicin (a, □—□) or ciprofloxacin 0.15 mg/L + sisomicin (b, □—□).

activities of nalidixic acid 50 mg/L or of ciprofloxacin 0.015 mg/L against *E. coli* KL16 in nutrient broth were also found to be unaffected by sub-inhibitory concentrations of the β -lactam cell-wall antagonists, ceftazidime, azlocillin and mezlocillin (tables I and II). Therefore, the bactericidal activity of the two 4-quinolones against *E. coli* KL16 appeared to be unaffected by sub-inhibitory concentrations of β -lactams or vancomycin.

The effects of sub-inhibitory concentrations of the same 16 antibacterials were also tested on a

third 4-quinolone, ofloxacin. Sub-inhibitory concentrations of all seven aminoglycosides tested enhanced the bactericidal activity of ofloxacin 0.06 mg/L against *E. coli* KL16 in nutrient broth (table III); all the 99% kill times were reduced. Conversely, sub-inhibitory concentrations of the other inhibitors of protein and RNA synthesis tested (rifampicin, tetracycline, chloramphenicol, erythromycin and clindamycin) increased the time required by ofloxacin 0.06 mg/L to kill 99% of the *E. coli* (table II) and hence antagonise the bacteri-

Fig. 6. Survival of *E. coli* KL16 in nutrient broth containing nalidixic acid 50 mg/L (a, ○—○), ciprofloxacin 0.015 mg/L (b, ○—○), vancomycin 0.4 mg/L (a and b, ●—●), nalidixic acid + vancomycin (a, □—□) or ciprofloxacin + vancomycin (b, □—□).

cidal activity of the 4-quinolone. As with ciprofloxacin and nalidixic acid, the four cell-wall antagonists tested had no effect on the bactericidal activity of ofloxacin against *E. coli* KL16 in nutrient broth. The results obtained with ofloxacin were, therefore, similar to those obtained with ciprofloxacin and nalidixic acid.

Discussion

The 16 antibacterials that were investigated for their effect on the bactericidal activities of ciprofloxacin, nalidixic acid and ofloxacin against *E. coli* KL16 in nutrient broth can be divided into three groups: firstly, the RNA synthesis inhibitor rifampicin and the protein synthesis inhibitors, tetracycline, erythromycin, clindamycin and chloramphenicol, that antagonised the bactericidal activity of the 4-quinolones; secondly, the aminoglycosides that enhanced 4-quinolone bactericidal activity; and thirdly the cell-wall antagonists that had no effect on 4-quinolone bactericidal activity. The aminoglycosides also enhanced the optimal bactericidal activity of the 4-quinolones. This is not always the case with combinations of antimicrobials, as enhancement may only occur at suboptimal drug concentrations, e.g., combinations of trimethoprim and sulphonamide (Amyes, 1982).

Unlike the other inhibitors of protein and RNA synthesis tested, the aminoglycosides damage the bacterial membrane (Tanaka, 1982; Davis, 1987). This is a secondary effect related to inhibition of protein synthesis, the addition of chloramphenicol having been shown to eliminate the membrane damage caused by streptomycin (Tanaka, 1982;

Tai and Davis, 1985). It has been suggested that this damage caused to the bacterial membrane by the aminoglycosides offers an explanation for these drugs being bactericidal against *E. coli* in contrast to chloramphenicol, tetracycline and erythromycin which are merely bacteriostatic (Tanaka, 1982; Tanaka *et al.*, 1984). The interaction between the aminoglycosides and the bacterial membrane would also seem to provide a possible explanation for the aminoglycosides forming a distinct sub-group amongst the inhibitors of protein synthesis in their interaction with the 4-quinolones. The membrane damage caused by the aminoglycosides, which is thought to be due to the incorporation of misread proteins into the membrane, increases the permeability of the membrane (Davis *et al.*, 1986; Davis, 1987) and also interferes with the initiation step of DNA synthesis (Tanaka *et al.*, 1984), probably by interfering with the formation of the *oriC*-membrane complex (Matsunaga *et al.*, 1986). These changes in membrane permeability may increase 4-quinolone uptake. Alternatively, the aminoglycosides may act *via* their effect on the initiation of DNA replication because the 4-quinolones also affect the replication of DNA. The observation that sub-inhibitory concentrations of aminoglycosides enhance the killing activity of the 4-quinolones, but not *vice versa* (Lewin, 1987), suggests that the first hypothesis is more probable. However, further investigation is necessary to identify the mechanism by which the aminoglycosides enhance 4-quinolone bactericidal activity.

Other workers have also demonstrated synergy between aminoglycosides and ciprofloxacin *in vitro* (Chalkley and Koornhof, 1985). It is interesting to

note that combinations of some cell wall antagonists and ciprofloxacin have been found to be synergic against *Pseudomonas aeruginosa* *in vivo* as well as *in vitro* (Chalkley and Koornhof, 1985; Giamarellou and Petrikos, 1987; Fu *et al.*, 1987; Bustamente *et al.*, 1987). Hence it is possible that the cell wall antagonists may, in some cases, act synergically with the 4-quinolones rather than the combination being additive or indifferent. However, no antagonism of the bactericidal activity of the 4-quinolones by the cell wall antagonists has been reported.

In conclusion, investigations on the effect of sub-inhibitory concentrations of other antibacterials on the bactericidal activity of nalidixic acid, ciprofloxacin and ofloxacin against *E. coli* KL16 in nutrient broth showed no antagonism between the 4-quinolones and cell wall antagonists. The aminoglycosides were found to form a distinct subgroup amongst the inhibitors of protein or RNA synthesis tested as the former enhanced the bactericidal activity of the 4-quinolones whilst the latter antagonised their bactericidal activity.

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