ANTIMICROBIAL AGENTS

The bactericidal activity of gemifloxacin (SB-265805)

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The bactericidal activity and mechanisms of action of the new fluoroquinolone gemifloxacin were investigated against the laboratory strains *Escherichia coli* KL16, *Staphylococcus aureus* E3T and *Streptococcus pneumoniae* C3LN4. Gemifloxacin was found to be highly bactericidal against these bacteria, producing a biphasic dose-response curve typical of the fluoroquinolones. This novel fluoroquinolone was more bactericidal than all other fluoroquinolones so far tested (ciprofloxacin, ofloxacin, enoxacin, lomefloxacin, levofloxacin, clinafloxacin, trovafloxacin, DV-7751 and sitafloxacin) against *S. aureus* and was more bactericidal than most other fluoroquinolones against *E. coli* or *Str. pneumoniae*. These data show gemifloxacin to be an improved member of the fluoroquinolone class of antibacterial agents.

Introduction

The bactericidal activity of numerous fluoroquinolones has been assessed in a series of studies [1]. This bactericidal activity has been characterised into four mechanisms of action (A, B, C and B₁) based on experiments investigating kill in phosphate-buffered saline (PBS), i.e., against non-multiplying bacteria, or kill in the presence of chloramphenicol (to prevent protein synthesis). Mechanism A requires bacteria to be multiplying and to be actively undertaking protein and RNA synthesis [2]. This is the basic mechanism of action shared by all quinolones and is the sole mechanism of action of older quinolones such as nalidixic acid. Mechanism B does not require multiplying bacteria or protein and RNA synthesis [2]. Mechanism B is shown by many modern fluoroquinolones against *Escherichia coli* [1]. However, this does not guarantee that this mechanism of action will be present against other bacteria. For example, ciprofloxacin does not possess mechanism B against *Staphylococcus aureus* [3], whereas levofloxacin does [4]. Against *Streptococcus pneumoniae*, only sitafloxacin has been found to possess this additional mechanism [1]. Mechanism C, on the other hand, does not require multiplying bacteria but does require active protein and RNA synthesis [1]. This mechanism has been identified

only with enoxacin [5] and norfloxacin [6]. Mechanism B₁ is the most recently discovered mechanism of action. This mechanism does not require protein or RNA synthesis, but is lost against non-dividing bacteria. Mechanism B₁ has been identified with clinafloxacin against *E. coli* or staphylococci [7] and with sitafloxacin or trovafloxacin against *Enterococcus faecalis* [1, 8].

This study assessed the bactericidal activity of gemifloxacin (SB-265805), a new (C-73-aminomethyl-4-methylimino-1-pyrrolidinyl substituted 1,8-naphthyridine) fluoroquinolone. Preliminary studies suggested that gemifloxacin has enhanced antibacterial activity, especially against gram-positive bacteria [9].

Materials and methods

Antimicrobial agents

Gemifloxacin was supplied by SmithKline Beecham Pharmaceuticals R&D (New Frontiers Science Park (South), Third Avenue, Harlow, Essex). Stock solutions of 1 g/L were prepared in sterile distilled water. Chloramphenicol (Sigma-Aldrich, Poole, Dorset) was first dissolved in methanol and then dissolved in sterile distilled water. Both antibacterial agents were prepared fresh on each day of experimentation.

Bacterial strains

The following laboratory strains were used in this study: *E. coli* KL16; *S. aureus* E3T; *Str. pneumoniae*

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C3LN4. These strains were chosen because of their use in earlier studies to determine the bactericidal mechanisms of action of other quinolones. The bacteria were stored at -70°C and subcultured on to Nutrient Broth No. 2 (Unipath, Basingstoke, Hants) solidified with bacteriological agar (Unipath) 1.5% w/v before use. For *Str. pneumoniae*, agar was supplemented with laked horse blood (Unipath) 5% v/v.

Bactericidal tests

The bactericidal activity of gemifloxacin was investigated by the method of Morrissey and Smith [1]. Briefly, a range of concentrations from 0.005 to 10 mg/L was prepared in nutrient broth no. 2. Bacteria were inoculated to an initial inoculum size of $c. 10^7$ cfu/L and incubated for 3 h at 37°C . When *Str. pneumoniae* was used, the medium was supplemented with laked horse blood to 7% v/v. The presence of additional mechanisms (B, B₁ or C) was assessed by the addition of chloramphenicol 20 mg/L (2.5 mg/L for *Str. pneumoniae*) to prevent protein synthesis or by replacing nutrient broth with PBS to prevent bacterial multiplication. To prevent autolysis of *Str. pneumoniae*, horse serum (Unipath) at 7% v/v was added when this organism was studied in PBS. After incubation, 1-ml samples were taken, centrifuged and resuspended in an equal volume of sterile nutrient broth no. 2. This washing step was repeated twice to prevent drug carry-over. Viable counts of these samples were made on solid agar by spiral plating and the plates were incubated for 48 h at 35°C . Percentage survival was calculated and plotted against drug concentration tested.

Results

Gemifloxacin produced a biphasic dose-response against *E. coli* KL16, *S. aureus* E3T and *Str. pneumoniae* C3LN4, producing an optimum bactericidal concentration for gemifloxacin against each test strain: *E. coli* KL16 1 mg/L, *S. aureus* E3T 0.5 mg/L and *Str. pneumoniae* C3LN4 0.5 mg/L (Fig. 1a,b,c). This phenomenon is typical of the fluoroquinolones [2].

The addition of a bacteriostatic concentration of chloramphenicol reduced the bactericidal activity of gemifloxacin against *E. coli* (Fig. 1a). However, some bactericidal activity still occurred in the presence of this inhibitor of protein synthesis. On the other hand, when the activity of gemifloxacin was tested in PBS, considerably less bactericidal activity was observed against *E. coli* (Fig. 1a). Therefore, it appears that gemifloxacin could kill bacteria devoid of protein synthesis but was less able to kill fully non-multiplying bacteria incubated in PBS. Therefore, gemifloxacin possesses bactericidal mechanisms A and B₁ against *E. coli*.

Slightly stronger bactericidal activity occurred against *S. aureus* in nutrient broth than that seen against *E. coli* (Fig. 1b). Reduced killing of the staphylococcus was seen when chloramphenicol was added or when experiments were performed in PBS (Fig. 1b). Nevertheless, good bactericidal activity still occurred against *S. aureus* E3T under these conditions, i.e., against staphylococci unable to undergo protein synthesis or against staphylococci unable to multiply. Therefore, it is evident that gemifloxacin possesses bactericidal mechanisms A and B against *S. aureus*.

Fig. 1. Survival of (a) *E. coli* KL16, (b) *S. aureus* E3T and (c) *Str. pneumoniae* C3LN4 treated with gemifloxacin for 3 h at 37°C : ●, nutrient broth (a,b) or blood broth (c; nutrient broth with laked horse blood 7%); ○, nutrient broth (a,b) or blood broth (c) plus chloramphenicol 20 mg/L; ■, PBS (a,b) or PBS with horse serum 7% (c).

Table 1. Comparison of bactericidal activity of gemifloxacin, trovafloxacin and sitafloxacin

Test strain	Quinolone	OBC (mg/L)	Percentage survival in			Reference no.
			NB	NB + Cm	PBS	
<i>E. coli</i> KL16	Gemifloxacin	1.0	0.004	2.5	12.3	This study
	Trovafloxacin	1.5	0.009	1.9	1.4	[8]
	Sitafloxacin	0.9	0.0005	0.028	0.07	[1]
<i>S. aureus</i> E3T	Gemifloxacin	0.5	0.0017	3.00	1.84	This study
	Trovafloxacin	3.0	0.005	0.16	0.44	[8]
	Sitafloxacin	3.0	0.004	0.09	0.18	[1]
<i>Str. pneumoniae</i> C3LN4	Gemifloxacin	0.5	0.21	16.2	25.7	This study
	Trovafloxacin	1.5	0.24	24.8	57.5	[8]
	Sitafloxacin	0.9	0.10	0.14	0.41	[1]

OBC, optimum bactericidal concentration; NB, nutrient broth; Cm, chloramphenicol.

It can be seen from Fig. 1c, that, as with the other two bacterial species tested, gemifloxacin was bactericidal against *Str. pneumoniae* in nutrient broth (supplemented with laked horse blood). This bactericidal activity was lower than that observed against either *E. coli* or *S. aureus*. Furthermore, little or no bactericidal activity occurred with gemifloxacin when chloramphenicol was added or when experiments were performed in PBS supplemented with horse serum. Therefore, against the pneumococcus, gemifloxacin did not possess any bactericidal mechanisms other than mechanism A.

Discussion

The results of this study show that gemifloxacin was bactericidal against *E. coli* KL16, *S. aureus* E3T and *Str. pneumoniae* C3LN4. This new fluoroquinolone possesses additional bactericidal mechanisms of action B₁ and B against *E. coli* KL16 and *S. aureus* E3T, respectively. However, no such additional mechanisms are present against *Str. pneumoniae* C3LN4.

For comparative purposes, the results of this study have been presented with the equivalent results obtained previously with trovafloxacin and sitafloxacin (Table 1). It can be seen that gemifloxacin has an optimum bactericidal concentration (OBC) similar to that of sitafloxacin and lower than that of trovafloxacin against *E. coli*. Gemifloxacin also has the lowest OBC against *Str. pneumoniae*. However, most significantly, gemifloxacin has a considerably lower OBC than trovafloxacin or sitafloxacin against *S. aureus*. Furthermore, the bactericidal activity of gemifloxacin at the OBC against *S. aureus* in nutrient broth was also greater than that found with the other fluoroquinolones (Table 1). These results show gemifloxacin to be the most potent fluoroquinolone so far tested against *S. aureus* E3T with this system.

However, it is interesting that the bactericidal advantage of gemifloxacin against *S. aureus* was not retained when chloramphenicol was added or when experiments were performed in PBS (Table 1). In other words,

bactericidal mechanism B with gemifloxacin was not as potent as that seen with trovafloxacin or sitafloxacin.

As seen with trovafloxacin (Table 1), gemifloxacin did not possess bactericidal activity in the presence of chloramphenicol or in PBS against *Str. pneumoniae*. In fact, sitafloxacin is the only fluoroquinolone known to possess bactericidal activity against *Str. pneumoniae* C3LN4 under these experimental conditions [1].

It is important to note that the strains used in this study are laboratory control strains – originally part of the culture collection at the School of Pharmacy, University of London. These strains were used for continuity and for comparison with previous investigations on the bactericidal mechanisms of action of quinolones. A similar but larger study of recent clinical isolates would be useful and may clarify the clinical significance of this and previous bactericidal investigations.

In conclusion, gemifloxacin showed reduced bactericidal activity when bacteria were unable to multiply due to weak additional mechanisms B or B₁ compared with trovafloxacin or sitafloxacin. Nevertheless, gemifloxacin showed very strong bactericidal activity against multiplying bacteria, especially against *S. aureus* E3T, compared with sitafloxacin and trovafloxacin. The clinical significance of these differences may become clear after further evaluation of gemifloxacin.

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