Antibacterial activity of a 1,8-naphthyridine quinolone, PD131628

C. S. LEWIN

Department of Medical Microbiology, University of Edinburgh Medical School, Teviot Place, Edinburgh EH8 9AG

Summary. A new 1,8-naphthyridine quinolone antimicrobial, PD131628, was found to be as, or more active than ciprofloxacin or ofloxacin against *Escherichia coli* and considerably more active than the two 4-quinolone agents against staphylococci in terms of both MIC and OBC (the optimum bactericidal concentration, at which the rate of kill is greatest). In common with ciprofloxacin and ofloxacin, the rate of kill of PD131628 against *Enterococcus faecalis* was considerably slower than against the other three species tested. Bacterial protein synthesis, RNA synthesis and cell division were not required for the bactericidal activity of PD131628 against *E. coli* or the staphylococci, although the lethality of PD131628 in the absence of these activities was reduced.

Introduction

The 4-quinolone antibacterial agents are rapidly bactericidal at concentrations above the minimum inhibitory concentration (MIC) and their rate of kill depends on several factors.^{1,2} Protein synthesis, RNA synthesis and bacterial cell division are essential for the bactericidal activity of older 4-quinolones such as nalidixic acid.^{1–3} One explanation for this is that the older 4-quinolones kill bacteria by a single mechanism, termed A, which requires bacterial protein and RNA synthesis as well as cell division.^{1–3} However, all the modern fluorinated 4-quinolones investigated so far are active against non-dividing bacteria, probably as a result of a bactericidal mechanism additional to A.³

The fluorinated 4-quinolones can be classified into two groups based upon the conditions required for their lethality. The first group, which includes ciprofloxacin, ofloxacin, DR-3355, lomefloxacin, pefloxacin and feroxacin, contains those whose second bactericidal mechanism, termed B, does not require bacterial protein or RNA synthesis.³ The second group, which includes norfloxacin and enoxacin, contains those that are bactericidal against non-dividing bacteria but still require protein and RNA synthesis to be able to kill bacteria, and this mechanism has been termed C.³

PD131628 (fig. 1), (s)-7-(3-amino-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1, 8-naphthyridine-3-carboxylic acid, is the active metabolite of the prodrug L-alanyl amide, PD131112. PD131628 differs from ciprofloxacin and ofloxacin in that it is a 1,8 naphthyridine, a chemical subclass of the quinolone antibacterial agents that demonstrates improved efficacy *in vivo* in animals and superior overall

pharmacokinetics in mice and man.⁴ PD131628 has low MICs against a wide range of gram-negative and gram-positive isolates,^{5,6} but determination of MIC does not provide any information on the conditions required for the killing of bacteria by a 4-quinolone. Furthermore, it does not provide any information on the optimum bactericidal concentration (OBC) of a 4-quinolone, defined as the concentration at which the rate of bacterial kill is greatest. Therefore, the conditions required for PD131628 to kill *Escherichia coli*, *Staphylococcus aureus*, *S. epidermidis* and *Enterococcus faecalis* and its ability to induce the SOS response, a DNA repair system associated with damage to bacterial DNA that is induced by other 4-quinolones,⁷ were determined. The activity of PD131628 against 60 blood culture isolates from the Royal Infirmary, Edinburgh, was also compared to that of ciprofloxacin and ofloxacin.

Materials and methods

Bacterial strains

Five test (collection) strains—*E. coli* KL16,³ *E. coli* GC4415,⁸ *S. aureus* E3T,⁹ *S. epidermidis* SK360⁹ and

Fig. 1. Structure of PD131628.

Ent. faecalis ATCC19433—were used in this study. They were chosen because of their previous use in evaluation of the bactericidal mechanisms of 4-quinolones. The strains were kept on drug-free nutrient agar plates and were sub-cultured every 10 days. Colonies taken from drug-free nutrient agar were used to prepare the overnight cultures used in these experiments. The clinical strains tested (20 *E. coli*, 20 *S. aureus* and 20 coagulase-negative staphylococci) were isolated during 1990 from blood cultures at the Royal Infirmary, Edinburgh, and were obtained from R. Paton.

Antibacterial preparations

Ofloxacin (Hoechst) was dissolved in 0.5 M NaOH (10.02 mg/ml) before being made up in sterile distilled water. Ciprofloxacin (Bayer), PD131628 (Parke-Davis), chloramphenicol (Parke-Davis) and rifampicin (Ciba) were dissolved in sterile distilled water.

Minimum inhibitory concentrations

MICs were determined by the serial dilution method in Isosensitest Agar (Oxoid) with spot inoculum of 10^4 cfu of each test strain. The MIC was taken as the lowest concentration that inhibited bacterial growth after overnight incubation of the plates at 37°C.

Determination of antibacterial effect of PD131628

The antibacterial activity of PD131628 was determined in Nutrient Broth (Oxoid) and in PBS at 37°C as previously described.¹⁰ Survival was estimated by serial dilution in nutrient broth followed by viable counts on nutrient agar.

Induction of the SOS response

Induction of the SOS response was measured as

previously described¹¹ with the modification that nutrient broth was substituted for LB Broth. SOS induction was expressed as β -galactosidase activity in Miller Units derived from the formula:

$$\frac{1000 \times <OD_{420} - 1.75 \times OD_{550}}{t \times v \times OD_{600}}$$

where OD_{420} and OD_{550} were the optical densities at the relevant wavelength after t min; v was the reaction volume and OD_{600} was the initial OD at 600 nm.

Results

The activity of PD131628 was equivalent to that of ciprofloxacin and greater than that of ofloxacin against the strains of *E. coli* tested (table). Against the 40 staphylococcal blood-culture isolates examined, PD131628 was five- to 10-fold more active than either of the clinically available 4-quinolones (table).

The bactericidal activity of PD131628 at a range of concentrations from 0.015 to 30 mg/L against *E. coli*, *S. aureus* and *S. epidermidis* in nutrient broth over 3 h showed a biphasic effect characteristic of the lethality of the 4-quinolones (fig. 2). Survival of bacteria decreased with increasing concentration of PD131628 until the OBC of 0.3 mg/L for all three species was reached and then survival increased progressively as the concentration rose above the OBC. Although the OBC was identical for all three species investigated, the level of kill observed at that concentration varied. PD131628 was 10-fold more active against *E. coli* than against the staphylococci. In contrast, after exposure for 3 h at 37°C, PD131628 was not bactericidal against *Ent. faecalis* ATCC19433 at concentrations over $100 \times$ MIC (fig. 2). However, after exposure for 6 h, PD131628 reduced enterococcal viability by one log, at concentrations two-fold or more above the MIC and at 24 h the amount of kill was similar to that seen

Table. MICs of quinolones for 60 blood-culture isolates from the Royal Infirmary, Edinburgh

Organism (number of strains)	Quinolone	Concentration (mg/L) of quinolone		
		MIC range	MIC50	MIC90
<i>E. coli</i> (20)	PD131628	<0.008–1	0.015	0.03
	Ciprofloxacin	<0.008–1	0.015	0.03
	Ofloxacin	0.06–2	0.12	0.25
<i>S. aureus</i> (20)	PD131628	0.015–0.25	0.06	0.12
	Ciprofloxacin	0.25–1	0.5	1
	Ofloxacin	0.25–1	0.5	1
Coagulase negative staphylococci (20)	PD131628	0.06–8	0.06	8
	Ciprofloxacin	0.25–16	0.25	8
	Ofloxacin	0.25–16	0.5	16

Fig. 2. Survival of *E. coli* KL16 ○—○, *S. aureus* E3T ●—●, *S. epidermidis* SK360 □—□ and *Ent. faecalis* ATCC19433 ■—■ after exposure for 3 h to PD131628 in nutrient broth at 37°C.

Fig. 3. Survival of *E. coli* KL16 in nutrient broth at 37°C with: PD131628 (0.3 mg/L) ●—●; PD131628 (0.3 mg/L)+chloramphenicol (20 mg/L) ■—■; PD131628 (0.3 mg/L)+rifampicin (160 mg/L) □—□; or chloramphenicol (20 mg/L) or rifampicin (160 mg/L) ○—○.

for the staphylococci after exposure for 3 h to the agent (results not shown). Hence, the rate of kill of PD131628 against *Ent. faecalis* was slower than against the other species tested.

At its OBC, PD131628 was still significantly bactericidal against *E. coli* in nutrient broth even in the presence of a bacteriostatic concentration of chloramphenicol, an inhibitor of protein synthesis, or rifampicin, an inhibitor of RNA synthesis (fig. 3). It can be seen from figs. 4 and 5 that the addition of a

Fig. 4. Survival of *S. aureus* E3T in nutrient broth at 37°C with: PD131628 (0.3 mg/L) ●—●; PD131628 (0.3 mg/L)+chloramphenicol (20 mg/L) □—□; or chloramphenicol (20 mg/L) ○—○.

Fig. 5. Survival of *S. epidermidis* SK360 in nutrient broth at 37°C with: PD131628 (0.3 mg/L) ●—●; PD131628 (0.3 mg/L)+chloramphenicol (20 mg/L) □—□; or chloramphenicol (20 mg/L) ○—○.

bacteriostatic concentration of chloramphenicol did not completely abolish the bactericidal activity of PD131628 at its OBC against *S. aureus* or *S. epidermidis*. However, the addition of chloramphenicol antagonised the lethality of PD131628 against the staphylococci considerably more than against *E. coli* (figs. 3–5). Less than 5% of the *E. coli* survived after exposure for 4 h to PD131628 with chloramphenicol, but 30% of the staphylococci survived after exposure for 4 h to both drugs. The bactericidal activity of PD131628 was then investigated in PBS. It can be seen in fig. 6 that PD131628 was able to kill *E. coli*, *S. aureus* and *S. epidermidis* under these conditions, although its bactericidal activity was considerably weaker against the staphylococci than against *E. coli*.

Inhibitory concentrations of PD131628 induced the SOS response in *E. coli* GC4415 after exposure for 1 h (fig. 7). The level of SOS induction initially rose with increasing concentration, peaked at 0.5 mg/L and then fell.

Fig. 6. Survival of (A) *S. epidermidis* SK360, (B) *S. aureus* E3T, and (C) *E. coli* KL16 in PBS ○—○ at 37°C and with PD131628 (0.3 mg/L) ●—●.

Fig. 7. Induction of SOS response in *E. coli* GC4415 exposed to PD131628 for 1 h at 37°C.

Discussion

In agreement with previously reported results,^{5,6} in comparison with ciprofloxacin or ofloxacin, PD131628 was considerably more active against the staphylococci and approximately equivalent in activity against *E. coli* in terms of MIC. The bactericidal activity of PD131628 against *E. coli*, *S. aureus* and *S. epidermidis*

showed the biphasic effect common to all other 4-quinolones so far investigated.³ The OBC of PD131628 was 0.3 mg/L for all three species. Therefore, PD131628 falls into the category of 4-quinolones that includes sparfloxacin and PD127391, whose OBCs are similar against the three species, rather than those, such as ciprofloxacin, whose OBCs are considerably higher against the staphylococci than against *E. coli*. In common with other 4-quinolones,^{12,13} PD131628 exerted a much slower rate of kill against the enterococci than against the staphylococci although the total amount of kill was similar after exposure for 24 h. Hence, PD131628 does not display more rapid bactericidal activity than ciprofloxacin or ofloxacin against the enterococci despite its greater activity in terms of MIC.^{5,6}

PD131628, like the other modern fluorinated 4-quinolones that have been investigated, displays a bactericidal mechanism additional to mechanism A. This appears to be mechanism B because PD131628 was able to kill bacteria in the absence of protein or RNA synthesis. It is interesting to note that PD131628 was considerably less bactericidal against the staphylococci than against *E. coli* when mechanism A was abolished, suggesting that the mechanism B displayed by the drug against the staphylococci was weaker than the mechanism B against *E. coli*. This correlates with a reduction in the amount of kill observed at the OBC for the staphylococci. This phenomenon has been observed for other fluoroquinolones and is most marked for ciprofloxacin, which does not appear to exert mechanism B against the staphylococci.¹⁴ However, as PD131628 still displays very high activity against the staphylococci in terms of OBC, despite the weak mechanism B, it would appear that the concentration at which the OBC is achieved is unaffected by the additional killing mechanism.

PD131628 induced the SOS response at concentrations above the MIC. The fluctuation in the level of induction of the SOS response by PD131628 has been reported with other 4-quinolones as well as with trimethoprim and zidovudine.^{7,15} The concentration at which maximum SOS induction occurred was in the region of the OBC, but the significance of this correlation must be questioned because induction of the SOS response does not appear to play a primary role in 4-quinolone lethality.^{7,10}

In conclusion, the activity of PD131628 was equivalent to, or greater than that of ciprofloxacin or ofloxacin against *E. coli* but considerably greater than that of either drug against the staphylococci. PD131628 therefore merits further investigation because its increased anti-staphylococcal activity may represent a significant improvement on the 4-quinolones that are currently available which are not always successful in treating staphylococcal infections.¹⁶

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