Bactericidal action of PD 127,391, an enhanced spectrum quinolone

C. S. LEWIN and S. G. B. AMYES

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

Summary. The 4-quinolone PD127,391 displays a biphasic effect on *Escherichia coli*, *Staphylococcus aureus* and *S. epidermidis* in nutrient broth. It is as active as ciprofloxacin in terms of its optimum bactericidal concentration against *E. coli*. However, against staphylococci it is six times as active as ciprofloxacin or any other 4-quinolone previously investigated. Although protein and RNA synthesis are not required for bactericidal activity, cell division is essential.

Introduction

PD127,391 is an enhanced spectrum, fluorinated 4-quinolone that exhibits low minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) against a wide range of gram-negative and gram-positive clinical isolates.^{1, 2}

The ability of the 4-quinolones to kill bacteria varies under different conditions.^{3, 4} Protein synthesis, RNA synthesis and cell division are essential for the bactericidal activity of older 4-quinolones such as nalidixic acid.^{3, 4} One explanation is that these kill bacteria by a mechanism, termed A, that requires protein synthesis, RNA synthesis and cell division.^{3–5} However, all the modern fluorinated 4-quinolones that have been investigated are active against non-dividing bacteria^{4, 6–9} probably because of a bactericidal mechanism other than A.³

The fluorinated 4-quinolones can be divided into two groups. In the first, which includes ciprofloxacin, ofloxacin,⁴ DR-3355,⁶ lomefloxacin,⁷ pefloxacin and fleroxacin,¹⁰ the second bactericidal mechanism, termed B, does not require bacterial protein and RNA synthesis. The second group contains norfloxacin and enoxacin which are bactericidal against non-dividing bacteria but require protein and RNA synthesis^{7, 8} for their additional mechanism, first found in norfloxacin and termed C.⁷

Determination of the MIC and MBC does not provide any information about the conditions required for the killing of bacteria by an antibacterial agent. Furthermore, these assays do not provide information on the optimum bactericidal concentration (OBC) at which killing activity is greatest. Therefore, we have studied the conditions required

for PD127,391 to kill *Escherichia coli*, *Staphylococcus aureus* and *S. epidermidis*.

Materials and methods

Bacterial strains

Laboratory strains *E. coli* KL16,³ *S. aureus* E3T¹¹ and *S. epidermidis* SK360¹² were used in this study. These strains were chosen because of their consistent use in previous evaluations of the bactericidal mechanisms of the 4-quinolones.^{4, 6–8, 11} The strains were kept on drug-free nutrient agar and subcultured every 10 days. Colonies taken from drug-free nutrient agar were used to prepare the overnight cultures used in these experiments.

Antibacterial preparation

PD127,391 (Parke-Davis, UK), chloramphenicol (Parke-Davis, UK) and rifampicin (Ciba, UK) were dissolved in sterile distilled water for incorporation in test media.

Determination of antibacterial effects of PD127,391

The killing activity of PD127,391 at drug concentrations of 0.005–9 mg/L was determined in nutrient broth (Oxoid) at 37°C over a 3-h period. Survival was estimated by serial dilution in nutrient broth followed by viable counts on nutrient agar as previously described.¹³ The rate of kill of bacteria exposed to PD127,391 in nutrient broth or phosphate-buffered saline (PBS) was measured at 30-min intervals over a 4-h period by viable counts on nutrient agar as previously described.¹¹

Results

The bactericidal activity of PD127,391 at concentrations of 0.005–9 mg/L against *E. coli*, *S. aureus* and *S. epidermidis* in nutrient broth over 3 h showed

a biphasic effect characteristic of the bactericidal activity of the 4-quinolones (fig. 1). The bactericidal activity increased until the OBC was reached. This was 0.15 mg/L for *E. coli* and 0.5 mg/L for both staphylococcal strains. At higher concentrations, the drug became less bactericidal. At its OBC, PD127,391 was still significantly bactericidal against *E. coli* in nutrient broth even in the presence of a bacteristatic concentration of chloramphenicol, which inhibits protein synthesis (fig. 2), or rifampicin, which inhibits RNA synthesis (fig. 3). The bactericidal activity of PD127,391 in the presence of chloramphenicol or rifampicin did not derive from residual protein or RNA synthesis because PD127,391 was still bactericidal when it was added to *E. coli* that had been pre-incubated for 60 min with either drug (figs. 2 and 3). However, when the two drugs were added together, some of the bactericidal activity of PD127,391 seemed to result from residual protein or RNA synthesis because

Fig. 1. Survival of *E. coli* (▲—▲), *S. aureus* E3T (○—○) and *S. epidermidis* SK360 (●—●) after exposure to PD127,391 in nutrient broth for 3 h at 37°C.

Fig. 2. Survival of *E. coli* KL16 in nutrient broth at 37°C with PD127,391 0.15 mg/L (○—○), PD127,391 0.15 mg/L + chloramphenicol 20 mg/L (■—■), chloramphenicol 20 mg/L + PD127,391 0.15 mg/L at 60 min (▲—▲) or chloramphenicol 20 mg/L (●—●).

the lethality of the 4-quinolone was reduced when it was added to the mixture at 60 min (figs. 2 and 3).

It can be seen from figs. 4 and 5 that the addition of a bacteristatic concentration of chloramphenicol did not completely abolish the bactericidal activity of PD127,391 at its OBC against *S. aureus* or *S. epidermidis*. The bactericidal activity did not result from residual protein or RNA synthesis because pre-incubation of the staphylococci with chloramphenicol did not reduce the bactericidal activity of the 4-quinolone (figs. 4 and 5). Hence PD127,391 seems to exert mechanism B, which does not require bacterial protein synthesis or RNA synthesis, against both *E. coli* and the staphylococci. Furthermore, the abolition of protein synthesis or RNA synthesis significantly reduced the bactericidal activity of PD127,391 against all three strains which confirms that the 4-quinolone possesses mechanism A.

The bactericidal activity of PD127,391 against *E. coli*, *S. aureus* and *S. epidermidis* was then investigated in PBS. It can be seen from fig. 6 that the 4-quinolone, at its OBC, was not significantly bactericidal against *E. coli* or *S. epidermidis* under

Fig. 3. Survival of *E. coli* KL16 in nutrient broth at 37°C with PD127,391 0.15 mg/L (●—●), PD127,391 0.15 mg/L + rifampicin 160 mg/L (■—■), rifampicin 160 mg/L + PD127,391 0.15 mg/L at 60 min (○—○) or rifampicin 160 mg/L (▲—▲).

Fig. 4. Survival of *S. aureus* E3T in nutrient broth at 37°C, with PD127,391 0.5 mg/L (○—○), PD127,391 0.5 mg/L + chloramphenicol 20 mg/L (▲—▲), chloramphenicol 20 mg/L + PD127,391 0.5 mg/L at 60 min (●—●) or chloramphenicol 20 mg/L (■—■).

Fig. 5. Survival of *S. epidermidis* SK360 in nutrient broth at 37°C, with PD127,391 0.5 mg/L (■—■), PD127,391 0.5 mg/L + chloramphenicol 20 mg/L (○—○), chloramphenicol 20 mg/L + PD127,391 0.5 mg/L at 60 min (▲—▲), or chloramphenicol 20 mg/L (●—●).

these conditions over a 4-h period. PD127,391 was slightly bactericidal against *S. aureus* in PBS (fig. 6) but the amount of kill was considerably less than that observed in nutrient broth containing chloramphenicol (fig. 4). Hence, bacterial cell division appears essential for the lethality of PD127,391.

Discussion

The bactericidal activity of PD127,391 against *E. coli*, *S. aureus* and *S. epidermidis* in nutrient broth showed a biphasic effect, common to other 4-quinolones.^{3, 4, 6-8, 10} The OBC of PD127,391 against *E. coli* (0.15 mg/L) is the same as that reported for ciprofloxacin³ and lower than reported for DR-3355 (0.5 mg/L),⁶ ofloxacin (0.9 mg/L),⁴ norfloxacin, lomefloxacin, pefloxacin, fleroxacin (all 1.5 mg/L)^{4, 7, 10} and enoxacin (3-5 mg/L).⁷ The OBC of PD127,391 against both *S. aureus* and *S. epidermidis* was 0.5 mg/L. This is significantly lower than that reported for the other 4-quinolones investigated. For example, the OBCs of the 4-quinolones with the most activity against staphylococci, DR-3355 and ciprofloxacin, are 3 mg/L for *S. aureus* and *S. epidermidis*.^{6, 11} This is six times

Fig. 6. A Survival of *E. coli* KL16 in PBS (●—●) and with PD127,391 0.15 mg/L (○—○) at 37°C.
 B Survival of *S. aureus* E3T in PBS (●—●) and with PD127,391 0.5 mg/L (○—○) at 37°C.
 C Survival of *S. epidermidis* SK360 in PBS (●—●) and with PD127,391 0.5 mg/L (○—○) at 37°C.

REFERENCES

- King A, Boothman C, Phillips I. The *in vitro* activity of PD127,139, a new quinolone. *J Antimicrob Chemother* 1988; **22**: 135–141.
- Wise R, Ashby JP, Andrews JM. *In vitro* activity of PD127,391, enhanced spectrum quinolone. *Antimicrob Agents Chemother* 1988; **32**: 1251–1256.
- Smith JT. Awakening the slumbering potential of the 4-quinolone antibiotics. *Pharm J* 1984; **233**: 299–305.
- Smith JT, Lewin CS. Chemistry and mechanisms of action of the quinolone antibacterials. In: Andriole VT (ed) *The quinolones*. London, Academic Press. 1988: 23–82.
- Deitz WH, Cook TM, Goss WA. Mechanism of action of nalidixic acid on *Escherichia coli*: III. Conditions required for lethality. *J Bacteriol* 1966; **91**: 768–773.
- Lewin CS, Amyes SGB. Bactericidal activity of DR-3355, an optically active isomer of ofloxacin. *J Med Microbiol* 1989; **30**: 227–231.
- Lewin CS, Amyes SGB, Smith JT. Bactericidal activity of enoxacin and lomefloxacin against *Escherichia coli* KL16. *Eur J Clin Microbiol* 1989; **8**: 731–733.
- Ratcliffe NT, Smith JT. Norfloxacin has a novel bactericidal mechanism unrelated to that of other 4-quinolones. *J Pharm Pharmacol* 1985; **37**: 92p.
- Zeiler H-J, Grohe K. The *in vitro* and *in vivo* activity of ciprofloxacin. *Eur J Clin Microbiol* 1984; **3**: 339–343.
- Lewin CS, Amyes SGB. Conditions required for the bactericidal activity of pefloxacin and feroxacin against *Escherichia coli* KL16. *J Med Microbiol* 1990; **32**: 83–86.
- Lewin CS, Smith JT. Bactericidal mechanisms of ofloxacin. *J Antimicrob Chemother* 1988; **22** Suppl C: 1–8.
- Tennent JM, Young H-K, Lyon BR, Amyes SGB, Skurray RA. Trimethoprim resistance determinants encoding a dihydrofolate reductase in clinical isolates of *Staphylococcus aureus* and coagulase-negative staphylococci. *J Med Microbiol* 1988; **26**: 67–73.
- Lewin CS, Howard BMA, Ratcliffe NT, Smith JT. 4-quinolones and the SOS response. *J Med Microbiol* 1989; **29**: 139–144.
- Lewin CS, Allen RA, Amyes SGB. Potential mechanisms of resistance to the modern fluorinated 4-quinolones. *J Med Microbiol* 1990; **31**: 153–162.

higher than that of PD127,391 against these strains. Hence, PD127,391 would appear to have an enhanced spectrum in terms of its bactericidal activity as well as its bacteristatic activity.²

PD127,391, like the other modern fluorinated 4-quinolones displays a bactericidal mechanism additional to mechanism A. This appears to be novel because cell division was essential for the lethal action of PD127,391. This contrasts with the other fluoroquinolones, all of which are active against non-dividing bacteria.^{3, 4, 7, 10} We propose that this mechanism be termed B₁ because it is similar to mechanism B³ in that it does not require protein synthesis or RNA synthesis to exert its lethal action.

In conclusion, PD127,391 seems to merit further investigation as, in contrast to the clinically available fluoroquinolones, it is almost as bactericidal against staphylococci as it is against *E. coli*. This enhanced activity against staphylococci may be significant because, at present, the 4-quinolones are not always successful in resolving staphylococcal infection and, indeed, resistance can develop during therapy.¹⁴

We are grateful to the Scottish Home and Health Department for financial support for CSL and to J. Matthews of Parke-Davis for providing us with PD127,391.