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The Fluoroquinolones Exert a Reduced Rate of Kill Against *Enterococcus faecalis*

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Abstract—The bactericidal activities of ciprofloxacin, ofloxacin and DR-3355 have been investigated against *Enterococcus faecalis*, *Staphylococcus aureus* and *Staphylococcus epidermidis* over 24 h. The three fluoroquinolones were found to be rapidly bactericidal against the staphylococci, killing over 99% of the bacteria during the first 3 h of exposure with a further reduction in viability of approximately one logarithm occurring over the next 21 h. In contrast, the fluoroquinolones displayed a much slower rate of kill against *E. faecalis*, as little or no bactericidal activity was detected over the first 3 h for both *E. faecalis* ATCC19433 and a clinical isolate. At 6 h all three of the drugs were bactericidal against the enterococci although the amount of kill was not as great as against the staphylococci. However, at 24 h the amount of kill obtained with all three drugs was similar to that obtained for staphylococci exposed to these drugs. Ciprofloxacin, ofloxacin and DR-3355 were not active against *E. faecalis* ATCC19433 in phosphate buffered saline and therefore require cell division for their bactericidal activity against this species.

The 4-quinolone antibacterials are useful for the treatment of urinary tract infections (UTIs) as the enterobacteriaceae and staphylococci, which cause the majority of such infections, are susceptible to these drugs (Phillips et al 1988). However, ciprofloxacin, ofloxacin and other fluoroquinolones in clinical use today are only marginally active against the enterococci which can cause up to 7·5% of significant hospital bacteriuria (Gruneberg 1984). For example, in Edinburgh, the minimum inhibitory concentration (MIC) of ciprofloxacin for 58 clinical enterococci isolates was found to be 2 mg L^{-1} (Paton et al 1989), slightly above the breakpoint of 1 mg L^{-1} proposed by a Working Party of the British Society of Antimicrobial Chemotherapy (1988). However, enterococcal UTIs should still respond to ciprofloxacin or ofloxacin therapy as these drugs attain urinary concentrations well above the MIC (Bergan 1988; Phillips et al 1988; Andrews et al 1990).

The fluoroquinolones display rapid bactericidal activity at concentrations just above the MIC against a wide range of species including enterobacteriaceae, *Ps. aeruginosa*, *S. marcescens* and staphylococci (Smith & Lewin 1988; Lewin et al 1991). However, when ciprofloxacin was tested against *E. faecalis* ATCC19433 and three clinical isolates of *E. faecalis* it was found to be merely bacteriostatic after 3 h exposure at 37°C in nutrient broth at concentrations as high as 150 × the MIC (Lewin et al 1989b). In urine, high levels of ciprofloxacin or other quinolones can be achieved for periods longer than 3 h (Bergan 1988). For example, the concentration of ciprofloxacin in urine 24 h after a single 500 mg oral dose is 10 mg L^{-1} (Gasser et al 1987). The bactericidal activities of ciprofloxacin, ofloxacin and DR-3355 (the *S*-(−)-isomer of ofloxacin) against *E. faecalis* ATCC19433 and a clinical isolate of *E. faecalis* were therefore investigated over 24 h. The ability of the three fluoroquinolones to kill non-dividing *E. faecalis* was also investigated.

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Materials and Methods

Bacterial strains

Staphylococcus aureus E3T, *S. epidermidis* SK360, *E. faecalis* ATCC19433 and *E. faecalis* CIP17, a clinical isolate obtained from R. Paton of the Royal Infirmary, Edinburgh, were used in this study. The strains were kept on drug-free nutrient agar plates which were subcultured every ten days. Colonies taken from drug-free nutrient agar plates were used to prepare the overnight cultures used in these experiments.

Antibacterial preparation

Ciprofloxacin (Bayer, UK) and DR-3355 (Daiichi Pharmaceutical Corp., Japan) were dissolved in sterile distilled water. Ofloxacin (Hoechst, UK) was dissolved in 0·5 M NaOH (50 mg mL^{-1}) before being made up to the appropriate concentration in sterile distilled water.

Determination of antibacterial effects of the 4-quinolones

The killing activity of the 4-quinolone at drug concentrations from 0·5 to 900 mg L^{-1} was determined in nutrient broth (Oxoid, UK) or phosphate-buffered saline (PBS) at 37°C over 24 h by the method of Lewin et al (1989a). Survival was estimated at 3, 6 and 24 h by serial dilution in nutrient broth followed by viable counting on nutrient agar as previously described (Lewin & Smith 1988) and expressed as percentage of initial culture viable count at each time period.

Results

Ciprofloxacin, at concentrations just above the MIC, was bactericidal against *S. aureus* E3T and *S. epidermidis* SK360 after 3 h exposure at 37°C. At the optimum bactericidal concentration (OBC), defined as the drug concentration causing the greatest killing of bacteria, over 99·9% of the *S. aureus* E3T (Fig. 1) and over 99% of the *S. epidermidis* SK360 (results not shown) were no longer viable after 3 h. A reduction in viability of a single logarithm or less occurred over the next 21 h. Similar results were obtained with ofloxacin and DR-3355 where the rate of kill of both drugs

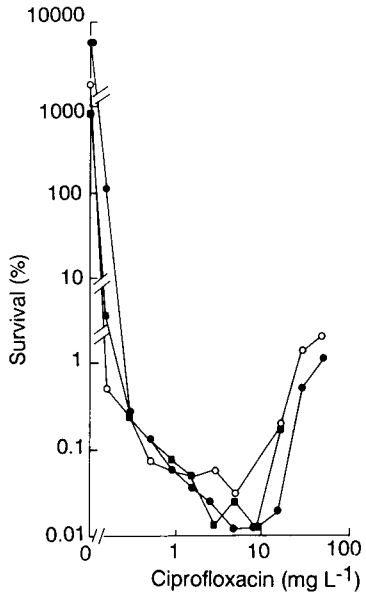

FIG. 1. Survival of *S. aureus* E3T exposed to ciprofloxacin at a range of concentrations in nutrient broth at 37°C. Percentage survival was estimated by viable counting on nutrient agar at 3 h (○), 6 h (●) and 24 h (■).

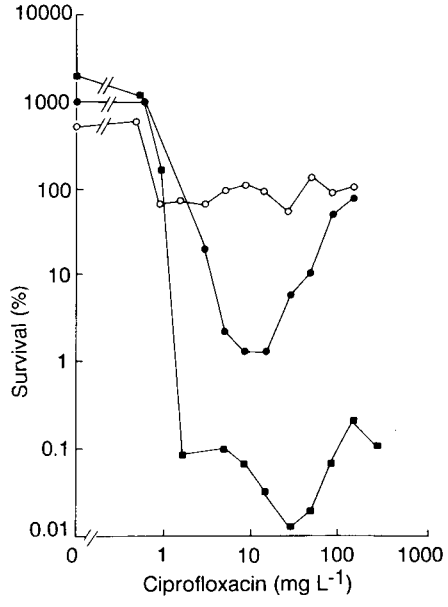

FIG. 2. Survival of *E. faecalis* ATCC19433 exposed to ciprofloxacin at a range of concentrations in nutrient broth at 37°C. Percentage survival was estimated by viable counting on nutrient agar at 3 h (○), 6 h (●) and 24 h (■).

against *S. aureus* E3T or *S. epidermidis* SK360 was greatest over the first 3 h of exposure to the drugs. Less than 1% of the bacteria survived 3 h exposure to the drugs at their respective OBCs and a further reduction in colony forming units per mL of approximately 1 logarithm occurred over the subsequent 21 h (results not shown). Against *E. faecalis* ATCC19433 ciprofloxacin was not significantly bactericidal after 3 h at concentrations of up to 150 mg L^{-1} (Fig. 2). After 6 h exposure ciprofloxacin was bactericidal as 95% of the enterococci were no longer viable at the OBC (Fig. 2). After 24 h exposure of *E. faecalis* ATCC19433 to ciprofloxacin, a decrease in viability similar to that obtained against the staphylococci was observed as less than 0·1% of the entero-

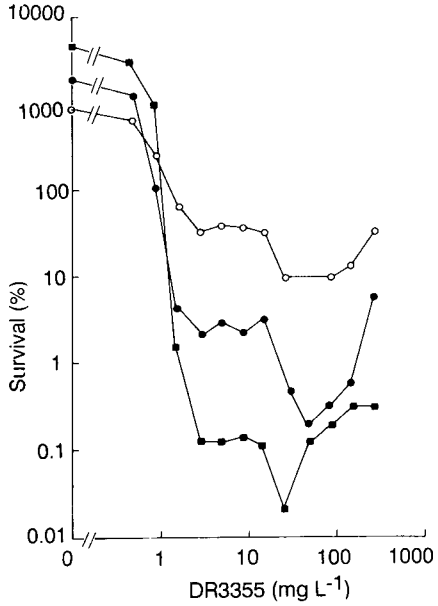

FIG. 3. Survival of *E. faecalis* ATCC19433 exposed to DR-3355 at a range of concentrations in nutrient broth at 37°C. Percentage survival was estimated by viable counting on nutrient agar at 3 h (○), 6 h (●) and 24 h (■).

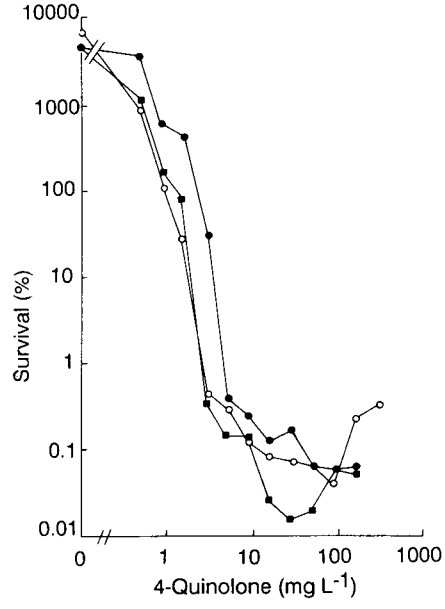

FIG. 4. Survival of *E. faecalis* CIP17 exposed to ciprofloxacin (○), DR-3355 (■) and ofloxacin (●) at a range of concentrations in nutrient broth at 37°C. Percentage survival was estimated by viable counting on nutrient agar at 24 h.

cocci survived 24 h exposure to ciprofloxacin at concentrations ranging from 1 to 100 mg L^{-1} (Figs 1, 2). Similarly, ofloxacin was not significantly bactericidal against *E. faecalis* ATCC19433 after 3 h exposure, was weakly bactericidal after 6 h exposure and highly bactericidal after 24 h exposure (results not shown). In contrast, DR-3355 was weakly bactericidal against *E. faecalis* ATCC19433 after 3 h exposure as up to 90% of the bacteria were killed at concentrations ranging from 30 to 150 mg L^{-1} (Fig. 3). DR-3355 was also more bactericidal than ofloxacin or ciprofloxacin at 6 h but at 24 h the bactericidal activity of all three fluoroquinolones was similar. Only 0·1% of the bacteria survived after

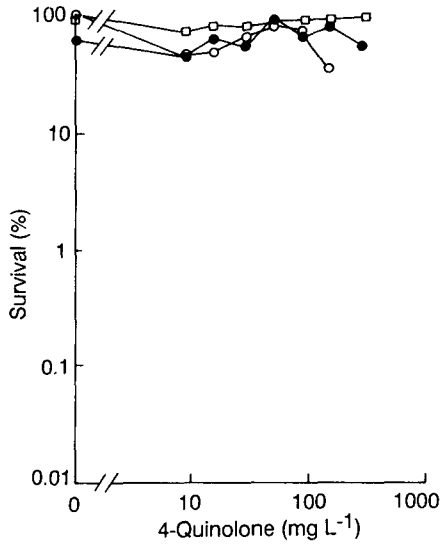

FIG. 5. Survival of *E. faecalis* ATCC19433 exposed to ciprofloxacin (○), DR-3355 (●) and ofloxacin (□) at a range of concentrations in PBS at 37°C. Percentage survival was estimated by viable counting on nutrient agar at 24 h.

24 h exposure to all three drugs at concentrations ranging from 5 to 150 mg L^{-1} (Figs 2, 3).

The bactericidal activities of the three drugs was also examined against *E. faecalis* CIP17, a clinical isolate. All three drugs displayed little bactericidal activity over the first 3 h of exposure (results not shown). The bactericidal activities increased over the next 21 h and at 24 h (Fig. 4), they were similar to that observed for the laboratory strain *E. faecalis* ATCC19433 (Figs 2, 3) as 0·1% or less of the bacteria were viable at the OBC.

The bactericidal activities of the three drugs at a range of concentrations were then examined against *E. faecalis* ATCC19433 in PBS where bacteria are unable to divide. *E. faecalis* ATCC19433 was found to be viable after 24 h exposure to ciprofloxacin, ofloxacin or DR-3355 in PBS at concentrations of up to 150 mg L^{-1} (Fig. 5). Hence, the three 4-quinolones were not bactericidal against non-dividing *E. faecalis*.

Discussion

The rate of kill of ciprofloxacin, ofloxacin or DR-3355 against *E. faecalis* was found to be significantly less than the rate of kill observed for all three drugs against the staphylococci or against enterobacteriaceae (Smith & Lewin 1988; Lewin & Amyes 1989). Against *S. aureus* and *S. epidermidis* most of the bactericidal activity shown by the three fluoroquinolones tested occurred during the first 3 h of exposure while against *E. faecalis* little or no bactericidal activity was detected over this time. Hence, not only are the 4-quinolones less active against the enterococci in terms of their ability to inhibit bacterial multiplication (Phillips et al 1988), they also exhibit a reduced rate of kill against this species at concentrations above the MIC. However, after 24 h exposure to the three drugs, the amount of kill obtained was similar for both the enterococci and the staphylococci. Thus, although the rate of kill is much slower against the enterococci, the fluoroquinolones are still just as bactericidal if the period of exposure is sufficiently long.

Ciprofloxacin, ofloxacin and DR-3355 were unable to kill non-dividing *E. faecalis*, which might offer an explanation for the slow rate of bactericidal activities of these drugs against this species. However, ciprofloxacin is unable to kill non-dividing staphylococci (Lewin & Smith 1988) but is just as rapidly bactericidal against this species as ofloxacin or DR-3355, which are bactericidal against non-dividing staphylococci (Lewin & Smith 1988; Lewin & Amyes 1989). The inability to kill non-dividing bacteria would therefore not seem to be the sole cause of the slow rate of kill by the three fluoroquinolones against *E. faecalis*. Further investigation is required to elucidate the factors involved in these differences.

Clinically, enterococcal UTIs should still respond to fluoroquinolone therapy despite the reduced rate of kill observed in this study. High concentrations of the fluoroquinolones should be maintained in urine long enough for bactericidal activity to occur (Bergan 1988). However, these results suggest that dosage should be maintained at high levels when treating such infections to ensure drug levels are adequate for eradication of the enterococci.

Acknowledgements

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