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Fleroxacin (Ro 23-6240): activity *in vitro* against 355 enteropathogenic and non-fermentative Gram-negative bacilli and *Legionella pneumophila*

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The antibacterial activity of fleroxacin (Ro 23-6240, AM-833), a new 6-fluoroquinolone, was determined against 149 strains of enteropathogenic bacteria (17 species) and 191 strains (28 species) of glucose non-fermentative Gram-negative rods (excluding *Pseudomonas aeruginosa*), and against 15 strains of *Legionella pneumophila*. The cumulative susceptibility of these groups of bacteria to Ro 23-6240 at the 2 mg/l level were 99·2%, 80·1 and 100% of tested strains, respectively.

Introduction

Fleroxacin (Ro 23-6240, AM-833), in common with other 6-fluoroquinolones, has been shown to be active against a wide range of Gram-positive and Gram-negative bacteria (Chin, Brittain & Neu, 1986; Hirai *et al.*, 1986). Up to now its activity against glucose non-fermentative bacteria and against many of the bacteria causing diarrhoea has remained undefined. The present study assesses the activity of Ro 23-6240 against these two diverse groups of potential pathogens, and against *Legionella pneumophila*.

Methods

Three hundred and fifty-five strains from the culture collection of the Department of Medical Microbiology of Zürich University and three control strains (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 29213) were used in this study. The origins of the glucose non-fermentative strains and the MIC techniques used in this study have been published previously (von Graevenitz & Bucher, 1982; National Committee for Clinical Laboratory Standards, 1983).

MICs were determined by standard microbroth serial two-fold dilution methods (NCCLS, 1983) in Mueller-Hinton broth (MHB) (0·1 ml) with an inoculum of approximately 5×10^4 cfu/well. MBCs were determined by standard methods (subculture volume 20 μl) and based on a 99·9% (MBC) reduction of the initial inoculum (Schoenknecht, Sabath & Thornsberry, 1985), as determined by initial and terminal colony counts for each strain.

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For fastidious strains, the methods and media used for susceptibility testing required alterations to standard techniques that have not yet been recognized by NCCLS. Hence, halophilic *Vibrio* spp. required the addition of 6·5% NaCl to broth for growth. For the testing of *L. pneumophila* an agar dilution technique with enriched charcoal-yeast extract agar was followed (Schoenknecht *et al.*, 1985) with incubation in air plus 10% CO_2 at 35°C for 48 h (Edelstein & Meyer, 1980). *Campylobacter jejuni* underwent agar dilution testing on Mueller-Hinton agar (MHA) plates (2·5% agar) enriched with 7% horse blood and incubation in an anaerobic jar with an activated gas generating system but without catalyst at 35°C over 48 h (Wang, Reller & Blaser, 1984). The control strains included in all such non-standard tests gave results consistent with those obtained in MHB.

Fleroxacin (Lot No. 0499720, potency 998 μg/mg) was obtained from an in-house source, solubilized in 1 ml of methanol and 0·06 ml sodium hydroxide 1 N in an ultrasonic bath and diluted in sterile water as needed.

Results

The results of the antibacterial susceptibility determinations of fleroxacin are displayed in Table I (aetiological agents of diarrhoea) and in Table II (glucose non-fermentative Gram-negative rods). The cumulative susceptibility of the Enterobacteriaceae and Vibrionaceae ($n = 129$) to fleroxacin was 99·2% at the $\leqslant 2$ mg/l level and 100% at the 4 mg/l level. The cumulative susceptibility of the 191 non-fermenters to fleroxacin was 80·1% at $\leqslant 2$ mg/l, 92·0% at $\leqslant 4$ mg/l, 98·8% at $\leqslant 8$ mg/l and 100% at the $\leqslant 16$ mg/l level. All *L. pneumophila* isolates were inhibited by 0·25 mg/l of fleroxacin.

The MBC of fleroxacin against bacteria causing diarrhoea was usually within one or two dilution steps of the corresponding MIC value. In contrast, for glucose non-

Table I. In-vitro activity of fleroxacin against causative bacterial agents of diarrhoea

Bacteria	Minimum inhibitory concentration (MIC) (mg/l)			Minimum bactericidal concentration (MBC) (mg/l)		
	MIC_{50}	MIC_{90}	Range	MBC_{50}	MBC_{90}	Range
E. coli ($N = 28$)	0·06	0·125	⩽0·06–4	⩽0·06	0·5	⩽0·06–8
Aeromonas spp. ($N = 20$)	⩽0·06	⩽0·06	⩽0·06–0·125	⩽0·06	⩽0·06	⩽0·06–0·125
Plesiomonas shigelloides ($N = 10$)	⩽0·06	⩽0·06	⩽0·06	⩽0·06	0·125	⩽0·06–0·25
Shigella spp. ($N = 20$)	⩽0·06	0·125	⩽0·06–0·25	⩽0·06	0·25	⩽0·06–0·5
Yersinia spp. ($N_{total}^{+} = 14$)	0·125	0·25	⩽0·06–2	0·125	0·25	⩽0·06–2
Y. pseudotuberculosis ($N = 3$)	⩽0·06	0·125	⩽0·06–0·125	0·25	0·25	⩽0·06–0·25
Y. kristensenii ($N = 1$)	⩽0·06	⩽0·06	⩽0·06	⩽0·06	⩽0·06	⩽0·06
Y. enterolitica ($N = 7$)	0·125	2	⩽0·06–2	0·25	2	⩽0·06–2
Y. frederiksenii ($N = 3$)	0·125	0·25	0·125–0·25	0·125	0·25	0·125–0·25
Salmonella spp. ($N = 22$)	⩽0·06	0·25	⩽0·06–0·5	⩽0·06	0·25	⩽0·06–0·5
Vibrio spp. ($N = 15$)	⩽0·06	0·125	⩽0·06–0·125	⩽0·06	0·125	⩽0·06–0·125
Camp. jejuni ($N = 20$)	0·25	1	0·25–1	–	–	–

Inoculum of 5×10^4 cfu/well

N_{total}^{+}, Sum of no. of the different *Yersinia* species listed immediately below N_{total}.

–, Not done (see Methods).

Table II. In-vitro activity of fleroxacin against glucose non-fermentative bacteria and *L. pneumophila*

Bacteria	Minimum inhibitory concentration (MIC) (mg/l)			Minimum bactericidal concentration (MBC) (mg/l)		
	MIC_{50}	MIC_{90}	Range	MBC_{50}	MBC_{90}	Range
Ps. maltophilia ($N = 10$)	4	4	2–8	8	16	4–16
Ps. acidovorans ($N = 5$)	0·25	2	⩽0·06–2	0·25	2	⩽0·06–2
Ps. alcaligenes ($N = 5$)	⩽0·06	0·5	⩽0·06–0·5	⩽0·06	0·5	⩽0·06–0·5
Ps. pseudoalcaligenes ($N = 7$)	0·25	16	⩽0·06–16	0·5	16	0·125–16
Ps. stutzeri ($N = 8$)	⩽0·06	0·5	⩽0·06–0·5	0·25	1	⩽0·06–1
Ps. pseudomallei ($N = 6$)	4	8	4–8	16	32	16–32
Ps. cepacia ($N = 9$)	4	8	2–8	8	16	2–16
Ps. putida ($N = 8$)	2	4	0·5–4	4	8	1–8
Ps. putrefaciens ($N = 4$)	0·25	1	⩽0·06–1	0·5	4	0·125–4
Ps. fluorescens ($N = 10$)	0·5	0·5	0·125–1	1	2	0·25–2
Ps. mendocina ($N = 3$)	⩽0·06	0·25	⩽0·06–0·25	0·25	0·5	⩽0·06–0·5
Ps. pickettii VA-2 ($N = 7$)	2	2	0·5–2	2	4	1–4
Ps. vesicularis ($N = 4$)	0·25	8	0·25–8	0·25	8	0·25–8
Ps. diminuta ($N = 6$)	4	8	4–8	4	8	4–8
Ps. VA-1 ($N = 7$)	2	2	0·5–2	4	4	0·5–4
Ps. VE ($N = 8$)	0·25	2	⩽0·06–2	0·25	2	⩽0·06–2
Flavobacterium II–B ($N = 8$)	1	4	0·5–4	1	4	0·5–4
F. multivorum ($N = 5$)	1	8	0·5–8	1	8	0·5–8
F. odoratum ($N = 6$)	2	4	2–4	32	64	4–64
F. meningosepticum ($N = 5$)	4	16	2–16	32	>128	4–>128
CDC Group II-F ($N = 6$)	2	4	0·5–4	8	64	2–64
Acinetobacter calcoaceticus						
subspecies *anitratus* ($N = 12$)	0·5	0·5	⩽0·06–0·5	0·5	1	⩽0·06–1
subspecies *lwoffii* ($N = 8$)	0·125	1	⩽0·06–1	0·5	2	⩽0·06–2
Alcaligenes denitrificans ($N = 4$)	2	4	⩽0·06–4	4	32	1–32
Alc. faecalis ($N = 4$)	⩽0·06	1	⩽0·06–1	0·125	16	⩽0·06–16
Alc. odorans ($N = 4$)	0·5	1	⩽0·06–1	1	2	1–2
Achromobacter VD ($N = 8$)	0·5	1	0·125–1	1	2	0·125–2
Ach. xylosoxidans ($N = 8$)	2	8	1–8	4	16	4–16
Moraxella urethralis ($N = 6$)	0·125	0·5	0·125–0·5	0·5	2	0·125–2
L. pneumophila ($N = 15$)[a]	0·125	0·25	0·06–0·25	–	–	–

Inoculum of 5×10^4 cfu/well.

[a]Agar dilution test using enriched CYE agar; incubation at 35°C over 48 h in CO_2. MBC test not available.

–, Not done.

fermentative rods the ninetieth percentile of the MBC was frequently identical to that of the MIC. However, for five of 29 species and subspecies tested with fleroxacin the MBC_{90} exceeded the MIC_{90} by three or more dilution steps. With application of a less stringent MBC definition that required the reduction of the inoculum 100-fold (rather than the customary 1000-fold), the resulting MBC_{90}s were mostly identical or lower by two than those reported above (data not shown).

The three control strains (*E. coli* ATCC 25922, *Staph. aureus* ATCC 29213, *Ps. aeruginosa* ATCC 27853) used in this trial yielded uniform MIC values to fleroxacin, with ranges of ⩽ 0·06, 0·5–1 and 2–4, respectively. These values were consistent with those found previously in our laboratory (unpublished data). Under the special conditions employed to test the activity of fleroxacin against *Camp. jejuni* and

L. pneumophila, the MIC values of *E. coli* ATCC 25922 were 0·06 mg/l and 0·25 mg/l, respectively.

Discussion

In the present study, fleroxacin proved to be active against Enterobacteriaceae and other bacterial species that cause diarrhoea. Fleroxacin—in common with other quinolones—was very potent against all enteropathogenic bacteria tested, possibly even more so weight for weight than all β-lactam agents other than imipenem, co-trimoxazole, tetracyclines and the two compounds with mainly intraluminal activity, bismuth subsalicylate and furazolidone. Fleroxacin concentrations of 2 or 4 mg/l (inhibitory against 99·2 and 100% of strains, respectively), have been shown to be clinically attainable in urine with a single oral dose of 400 mg and in plasma and tissue fluid with an oral dose of 800 mg (Weidekamm, Stöckel & Dell, 1987). Against the commonly encountered enteropathogens our results with fleroxacin are consistent with those of other authors (Carlson *et al.*, 1983; Fass, 1985; Chin *et al.*, 1986) who have evaluated the new quinolones.

The activity of fleroxacin was not influenced by production (9 strains) or not (3 strains) of enterotoxin in *E. coli*. The compound was invariably active against *Shigella sonnei* (7), *Sh. flexneri* (7) and *Sh. boydii* (5), as well as *Sh. dysenteriae* (1). Non-typhi salmonellae (13) were as susceptible as *Salmonella typhi* (9).

No noteworthy differences emerged between the different *Vibrio* spp. tested (*V. cholerae* (5), *V. parahaemolyticus* (5) and *V. alginolyticus* (5)). Of the *Yersinia* spp., one isolate of *Y. enterocolitica* (7) was at least eight-fold more resistant than the others. Some of the more unusual species tested have only recently been recognized as enteropathogenic bacteria, i.e. *Y. kristensenii* (1) (Baier & Ruppel, 1981), *Y. frederiksenii* (3) (Baier & Puppel, 1981), *Y. pseudotuberculosis* (3) (Tertti *et al.*, 1984), *V. alginolyticus* (5) (Joseph, Colwell & Kaper, 1982), *V. parahaemolyticus* (5) (Joseph *et al.*, 1982) and *Aeromonas hydrophila* (Holmberg & Farmer, 1984). For reasons not well understood, all quinolones including fleroxacin (data on file) are as inactive against *Clostridium difficile* (Fass, 1985) as they are against many other anaerobic bacilli.

On the strength of the antibacterial activity demonstrated, fleroxacin should be effective in the treatment and prophylaxis of both diarrhoea and septicaemias caused by *Salmonella* spp. or *Campylobacter* spp. Comparative trials with erythromycin and a placebo against *Camp. jejuni* should determine the place of fleroxacin in *Camp. jejuni* infections. Likewise, clinical trials with fleroxacin and standard comparative compounds and/or placebo should be undertaken in all forms of gastrointestinal disease due to other enteropathogenic bacteria susceptible to fleroxacin *in vitro*. Indeed, the combination of high systemic and intraluminal activity of quinolones suggests that the prophylaxis and treatment of gastrointestinal infections could be a major indication for fleroxacin and its congeners.

The present study also provides new information on the activity of fleroxacin against glucose non-fermentative Gram-negative bacteria. Although it is about eight-fold less active on a weight-by-weight basis than ciprofloxacin (99% of isolates susceptible to $\leqslant 1$ mg/l; Husson *et al.*, 1985), the higher plasma levels of fleroxacin and the lack of side effects for single doses of up to 1500 mg orally in volunteers (Weidekamm *et al.*, 1987) imply that fleroxacin could have a role in the treatment of infections brought

about by these unusual pathogens, particularly if they involve the urinary tract. However, ciprofloxacin is also very active against these same organisms (Baier & Puppel, 1981; Grimm, 1985)). Ultimately, more information on the side effects of fleroxacin as well as a comparative in-vitro study will be needed to decide whether fleroxacin or ciprofloxacin is tolerated better and is more active *in vivo* (Neu & Ellner, 1983) against these isolates.

An additional advantageous feature of fleroxacin was that its bactericidal activity remained within one or two dilution steps of its inhibitory activity for all but a few strains. This asset—if clinically significant—could prove especially useful against non-fermentative organisms in the immunosuppressed. This finding is not unique to fleroxacin but is shared by all 6-fluoroquinolones.

Fleroxacin was also active *in vitro* against *L. pneumophila*. There is experimental evidence that this might be clinically significant because quinolones including fleroxacin are known to diffuse into murine macrophages where they attain levels two- to three-fold higher than in serum or tissue fluids (Easmon & Crane, 1985).

All the above findings on fleroxacin combined with its high bactericidal activity, the fact that it is well tolerated and has an elimination half-life ($T_{1/2}\beta$) in excess of 9 h suggest that this new quinolone possesses features that are desirable in any new antibacterial about to enter clinical evaluation.

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