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Faropenem, a new oral penem: antibacterial activity against selected anaerobic and fastidious periodontal isolates

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The *in vitro* activity of faropenem, an oral penem, was compared with those of penicillin, co-amoxiclav, cefoxitin, clindamycin, erythromycin and metronidazole against 106 isolates of anaerobic pathogens involved in systemic infections. The organisms tested comprised *Porphyromonas gingivalis* (29), *Prevotella* spp. (eight), *Prevotella melaninogenica* (seven), *Prevotella intermedia* (five), *Actinomyces* spp. (25), *Fusobacterium nucleatum* (14), *Peptostreptococcus* spp. (11), *Bacteroides ureolyticus* (five) and *Bacteroides forsythus* (two). The antimicrobial properties of faropenem were investigated by studying MICs, MBCs, time–kill kinetics and post-antibiotic effect (PAE). Faropenem was highly active against all the anaerobes tested (MIC_{90} ≤ 0.5 mg/L) and was bactericidal against both β-lactamase-positive and -negative anaerobes, with a maximum bactericidal effect at 10 × MIC at between 12 and 24 h. In addition, faropenem had an *in vitro* PAE on all the tested isolates and this was not influenced by β-lactamase production. Faropenem may be useful for treating infections caused by periodontal bacteria or oral flora.

Keywords: faropenem, anaerobic pathogens, antibacterial activity

Introduction

Periodontal anaerobic pathogens are often associated with various infections, including systemic illnesses such as bacteraemia, endocarditis, brain abscesses, urogenital, skin and soft tissue, pulmonary, gastrointestinal and urogenital infections.[1,2] The different susceptibilities of these pathogens to antimicrobial agents make therapy very difficult.[3,4]

Faropenem is a unique antimicrobial penem being developed for oral administration as the pro-drug ester, faropenem-daloxate. Penems share structural similarities with both penicillins and cephalosporins, and are characterized by a broad antibacterial spectrum, a potent penicillin-binding protein affinity and good β-lactamase stability.[5–7]

The aim of this study was to evaluate the antibacterial activity of faropenem in comparison with that of other antibiotics against anaerobic pathogens involved in systemic infections, and to evaluate the *in vitro* pharmacodynamic properties of faropenem by studies of time–kill kinetics and post-antibiotic effect (PAE).

Materials and methods

Microorganisms

We tested 106 recent isolates collected from periodontal infections: *Porphyromonas gingivalis* (29), *Prevotella* spp. (eight), *Prevotella melaninogenica* (seven), *Prevotella intermedia* (five), *Actinomyces* spp. (25), *Fusobacterium nucleatum* (14), *Peptostreptococcus* spp. (11), *Bacteroides ureolyticus* (five) and *Bacteroides forsythus* (two). The identification of bacteria was made by colony and cellular morphology, staining characteristics, motility test and biochemical tests.[8,9] Further bacterial identifications were carried out using API 20A, API-ZYM and rapid ID32A (bioMérieux).

All isolates with penicillin MICs ≥ 0.25 mg/L were tested for production of β-lactamase by spreading fresh (24–48 h) cultures on filter paper moistened with 500 mg/L nitrocefin.

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Antibiotics

The antibacterial activity of faropenem was studied in comparison with that of penicillin G (Pharmacia), co-amoxiclav (SmithKline Beecham), cefoxitin (Merck, Sharp & Dôhme), erythromycin (Abbott), clindamycin (Upjohn) and metronidazole (Bristol-Myers Squibb). The agents tested, as powders of known potency, were gifts from their respective manufacturers.

Determination of MICs and MBCs

MICs of faropenem and of the other antimicrobials in the comparison were determined by the microdilution method using Brucella broth supplied with haemin (0.005 mg/L) and vitamin K_1 (0.0004 ml/L) with an inoculum of 10^5 cfu/mL, in accordance with the guidelines of the NCCLS.[10] *Bacteroides fragilis* ATCC 25285 was used as a control strain. The MIC was defined as the lowest concentration at which there was no visible growth after incubation at 35–37°C for 48 h.

The susceptibility breakpoints recommended by the NCCLS for anaerobic bacteria were: penicillin ≤ 0.5 mg/L, co-amoxiclav ≤ 4/2 mg/L, cefoxitin ≤ 16 mg/L, clindamycin ≤ 2 mg/L and metronidazole ≤ 8 mg/L. For erythromycin, the value of ≤0.5 mg/L was used according to the recommendations for aerobic bacteria.[11] NCCLS breakpoints for faropenem are not yet available.

After the determination of faropenem MICs, subcultures onto supplemented Brucella agar were obtained from the wells devoid of growth by means of a 6 mm loop. Incubation was carried out at 35–37°C in anaerobic jars for 48 h. The MBC was defined as the lowest antibiotic concentration resulting in no visible colony growth.

Time–kill kinetics

The killing curves of faropenem and co-amoxiclav were carried out in accordance with the method of Rosenblatt,[12] against two isolates each of *P. gingivalis*, *B. ureolyticus* and *Actinomyces* spp. and one isolate of *Prevotella* sp. and *F. nucleatum.* In a closed system the bacteria were incubated with faropenem at concentrations equivalent to the MIC, 4 × and 10 × MIC, and with co-amoxiclav at MIC and 4 × MIC. Bactericidal activity was defined as a 3 $\log_{10}$ decrease (99.9% kill) in cfu/mL.

PAE

The PAE of faropenem was determined by the viable plate count method of Craig & Gudmundsson[13] against eight isolates of periodontal bacteria: two isolates each of *P. gingivalis* (CT 12 β-lac+, MIC 0.125 mg/L and CT 23 β-lac–, MIC 0.25 mg/L), *B. ureolyticus* (CT 21 β-lac–, MIC 0.06 mg/L and CT 42 β-lac+, MIC 0.06 mg/L), *Actinomyces* sp. (CT 10 β-lac–, MIC 0.06 mg/L and CT 25 β-lac–, MIC 0.125 mg/L), *Prevotella* sp. (CT 7 β-lac–, MIC 0.06 mg/L) and *F. nucleatum* (CT 2 β-lac–, MIC 0.125 mg/L).

The PAE was determined in duplicate on supplemented Brucella agar at faropenem concentrations of 4 × and 10 × MIC. A significant PAE was defined as an effect of >0.5 h.

Results and discussion

The *in vitro* activities of the drugs tested against 106 isolates are shown in Table 1.

β-Lactamase production in isolates of *B. forsythus* (50%), *B. ureolyticus* (20%), *Prevotella* spp. (20%), *F. nucleatum* (7%) and *P. gingivalis* (3%) compromised the activity of penicillin (MIC ≥ 1 mg/L). Faropenem had significant activity (MIC_{90} ≤ 0.5 mg/L) against both β-lactamase-producing and -non-producing isolates. *B. forsythus* isolates were inhibited by faropenem concentrations of 0.06 and 0.12 mg/L, and *B. ureolyticus* by concentrations ranging from ≤0.03 to 0.5 mg/L. β-Lactamase-positive isolates remained susceptible to co-amoxiclav and cefoxitin (MIC ≤ 4 mg/L). Erythromycin was generally less active than faropenem, in particular against *F. nucleatum*, *Peptostreptococcus* spp., *B. forsythus* and *B. ureolyticus*. Good activity was observed for clindamycin, particularly against *Prevotella* spp. (MIC range ≤0.03–2 mg/L) and *F. nucleatum* (MIC range ≤0.06–0.25 mg/L). The majority of all tested species were inhibited by metronidazole at ≤8 mg/L, except for *Actinomyces* spp. (MIC range 8–>64 mg/L).

Since breakpoint interpretative criteria for faropenem have not been established for anaerobic bacteria, our study does not report percentage susceptibility. The overall percentage of isolates susceptible to penicillin was 91%. Except for *Peptostreptococcus* spp. (91%), all the species tested were susceptible to co-amoxiclav and cefoxitin (100%). The percentage of susceptibility to clindamycin of all isolates tested was 95%, whereas the susceptibility to metronidazole was 100%, except for *Actinomyces* spp. (34%). Using the value of ≤0.5 mg/L, according to the recommendations for aerobic bacteria,[12] the overall susceptibility percentage to erythromycin was 77%; however, the activity of erythromycin can be affected by anaerobic conditions, mainly when fusobacteria are tested.

MBCs of faropenem were equal to or 2–4 × higher than MICs.

Against the isolates of *P. gingivalis* and *Actinomyces* spp. (Figure 1a–d), faropenem exhibited a bactericidal activity at 10 × MIC at 12 h, whereas against *B. ureolyticus* and *F. nucleatum* (Figure 1e–g) a bactericidal effect was observed at 24 h.

Faropenem had an efficient killing activity against *Prevotella* spp. (Figure 1h) at both 10 × and 4 × MIC, which compared favourably with that of co-amoxiclav.

For *P. gingivalis* and *Actinomyces* spp. isolates, co-amoxiclav at 10 × MIC was bactericidal at 12 h (Figure 1a–d).

Table 1. Antibacterial activity of faropenem compared with other antibiotics against 106 periodontal anaerobic isolates

Isolates (n^a)	Antimicrobial agents	% S	MIC_{50} (mg/L)	MIC_{90} (mg/L)	MIC range (mg/L)	MBC range (mg/L)
P. gingivalis	faropenem	–	0.06	0.5	≤0.03–0.5	0.5–1
29 (1)	penicillin	97	≤0.03	0.06	≤0.03–1	–
	co-amoxiclav	100	≤0.03	0.06	≤0.03–0.06	–
	cefoxitin	100	0.12	0.5	0.06–0.5	–
	erythromycin	93	0.06	0.25	≤0.03–8	–
	clindamycin	93	≤0.03	0.06	≤0.03–16	–
	metronidazole	100	0.06	2	≤0.03–2	–
Prevotella spp.[b]	faropenem	–	0.06	0.5	≤0.03–0.5	0.5–1
20 (4)	penicillin	80	0.25	2	≤0.03–32	–
	co-amoxiclav	100	0.06	1	≤0.03–2	–
	cefoxitin	100	0.25	2	0.12–4	–
	erythromycin	85	0.25	1	0.25–4	–
	clindamycin	100	≤0.03	2	≤0.03–2	–
	metronidazole	100	0.5	1	0.12–2	–
Actinomyces sp.	faropenem	–	0.06	0.5	≤0.03–2	0.5–1
25	penicillin	92	0.06	0.12	≤0.03–32	–
	co-amoxiclav	100	0.12	0.5	0.06–2	–
	cefoxitin	100	0.12	1	0.06–2	–
	erythromycin	88	0.12	1	0.06–32	–
	clindamycin	92	0.12	0.5	0.06–32	–
	metronidazole	34	16	>64	8–>64	–
F. nucleatum	faropenem	–	0.12	0.25	≤0.03–0.5	0.5–2
14 (1)	penicillin	93	≤0.03	≤0.06	≤0.03–32	–
	co-amoxiclav	100	≤0.03	0.06	≤0.03–0.5	–
	cefoxitin	100	0.06	0.12	≤0.03–2	–
	erythromycin	43	4	16	2–16	–
	clindamycin	100	≤0.03	0.12	≤0.03–0.25	–
	metronidazole	100	0.06	0.12	≤0.03–0.5	–
Peptostreptococcus sp.	faropenem	–	0.06	0.12	≤0.03–16	0.12–0.25
11	penicillin	91	≤0.03	0.25	≤0.03–16	–
	co-amoxiclav	91	0.12	0.25	≤0.03–16	–
	cefoxitin	91	0.5	0.5	≤0.03–16	–
	erythromycin	45	1	16	≤0.03–>64	–
	clindamycin	90	0.5	4	≤0.03–>64	–
	metronidazole	100	1	2	≤0.03–8	–
B. forsythus	faropenem	–	–	–	0.06–0.12	0.12–0.25
2 (1)	penicillin	50	–	–	≤0.03–1	–
	co-amoxiclav	–	–	–	0.06–0.12	–
	cefoxitin	–	–	–	0.12–0.5	–
	erythromycin	50	–	–	0.5–2	–
	clindamycin	–	–	–	0.06–0.12	–
	metronidazole	–	–	–	0.12–0.25	–
B. ureolyticus	faropenem	–	–	–	≤0.03–0.5	0.12–0.5
5 (1)	penicillin	80	–	–	≤0.03–1	–
	co-amoxiclav	80	–	–	≤0.03–2	–
	cefoxitin	–	–	–	0.06–1	–
	erythromycin	40	–	–	0.06–4	–
	clindamycin	80	–	–	≤0.03–4	–
	metronidazole	–	–	–	0.12–1	–

[a]Numbers of β-lactamase-positive isolates are shown in parentheses.
[b]*Prevotella* spp. (eight), *P. melaninogenica* (seven), *P. intermedia* (five).

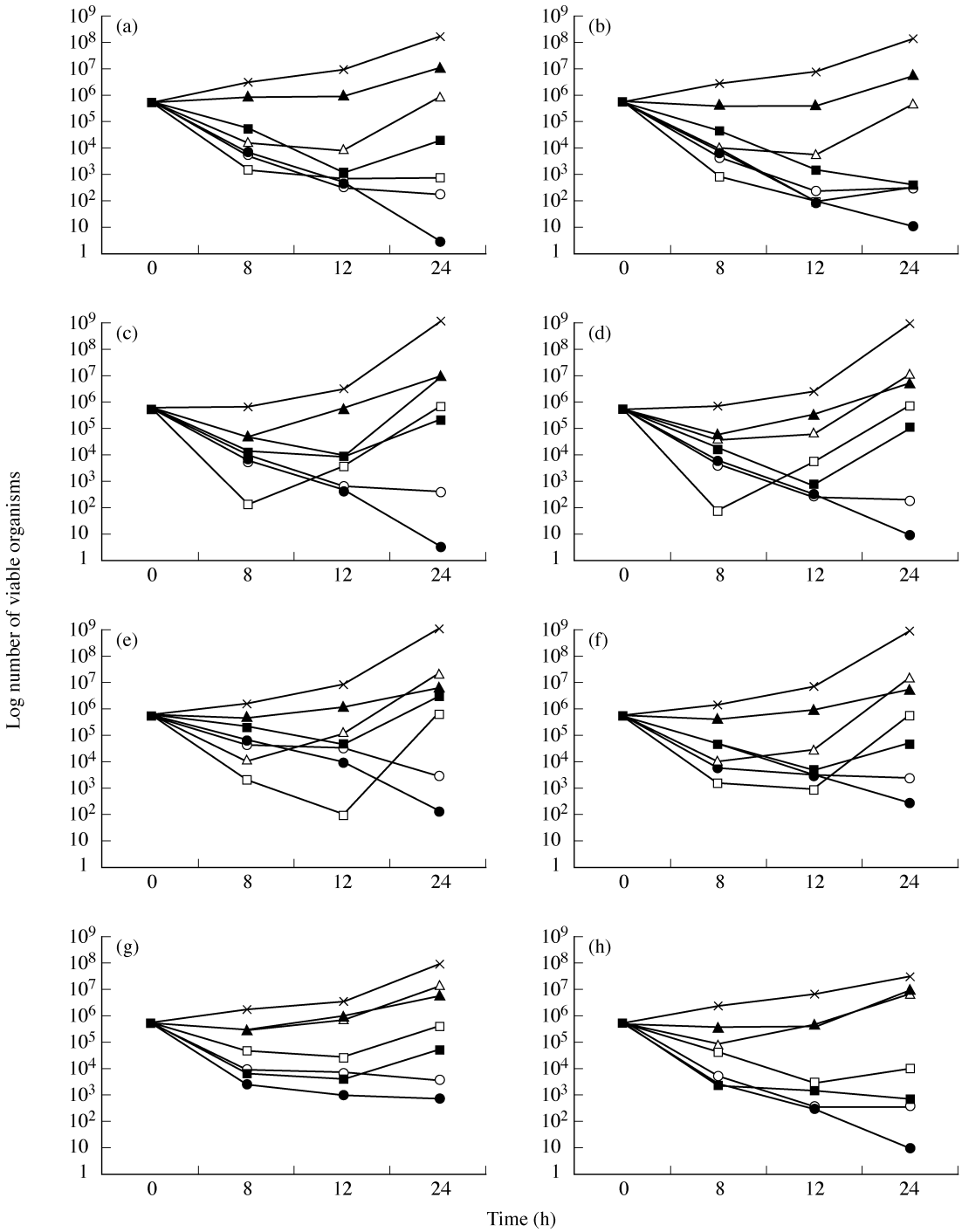

Figure 1. Killing curves of faropenem (F) (black triangles, MIC; black squares, 4 × MIC; black circles, 10 × MIC) and co-amoxiclav (A/C) (white triangles, MIC; white squares, 4 × MIC; white circles, 10 × MIC) against (a) *P. gingivalis* CT 12 β-lac+ (F MIC, 0.125 mg/L and A/C MIC, 0.5 mg/L), (b) *P. gingivalis* CT 23 β-lac– (F MIC, 0.25 mg/L and A/C MIC, 0.5 mg/L), (c) *Actinomyces* sp. CT 10 β-lac– (F MIC, 0.06 mg/L and A/C MIC, 0.5 mg/L), (d) *Actinomyces* sp. CT 25 β-lac– (F MIC, 0.125 mg/L and A/C MIC, 0.5 mg/L), (e) *B. ureolyticus* CT 42 β-lac+ (F MIC, 0.06 mg/L and A/C MIC, 0.5 mg/L), (f) *B. ureolyticus* CT 21β-lac– (F MIC, 0.06 mg/L and A/C MIC, 0.5 mg/L), (g) *F. nucleatum* CT 2 β-lac– (F MIC, 0.125 mg/L and A/C MIC, 0.5 mg/L), (h) *Prevotella* sp. CT 7 β-lac– (F MIC, 0.06 mg/L and A/C MIC, 0.125 mg/L); control curves, crosses.

At 4 × MIC, co-amoxiclav exerted a bactericidal effect at 8 h against *Actinomyces* spp. (Figure 1c and d) and at 12 h against *B. ureolyticus* CT 42 (Figure 1e).

Unlike most β-lactams, faropenem exhibited an *in vitro* PAE on some clinically relevant anaerobic bacteria, and this effect was not influenced by β-lactamase production.[13]

Table 2. Faropenem post-antibiotic effect (PAE)

Isolates	MIC (mg/L)	PAE (h)	
		4 × MIC	10 × MIC
P. gingivalis CT 12	0.125	2.38	2.72
P. gingivalis CT 23	0.25	2.45	2.87
Prevotella sp. CT 7	0.06	2.21	2.56
Actinomyces sp. CT 10	0.06	0.33	1.00
Actinomyces sp. CT 25	0.125	0.52	1.18
F. nucleatum CT 2	0.125	1.75	1.96
B. ureolyticus CT 21	0.06	1.50	2.00
B. ureolyticus CT 42	0.06	1.82	2.27

Generally, increasing concentrations of faropenem were associated with increases in the PAE. The PAE was marked for *P. gingivalis* both at 4 × and 10 × MIC. However, in all cases the greatest effect was observed at 10 × MIC. The shortest PAE was observed for *Actinomyces* spp. at both 4 × and 10 × MIC (Table 2). The differences in PAE duration at 4 × and 10 × MIC of faropenem were not significant, except for *Actinomyces* spp., against which 10 × MIC produced a PAE two to three times longer than that obtained at 4 × MIC.

Our results show that faropenem has potent antibacterial activity against periodontal pathogens comparable to that of cefoxitin and co-amoxiclav, and higher than that of clindamycin and metronidazole. The time–kill kinetics showed that faropenem was bactericidal against both β-lactamase-positive and -negative anaerobic bacteria, and appeared to exhibit time-dependent bactericidal activity, which is usual for β-lactams. The performance of faropenem in the present study, in addition to its broad spectrum of activity and its stability to β-lactamases, makes it a promising new antimicrobial agent in anaerobic or mixed infections.

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