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Comparative In Vitro Activities of Cefpiramide and Apalcillin Against Anaerobic Bacteria

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The in vitro activities of two new antimicrobial agents, apalcillin and cefpiramide (SM-1652), were evaluated against 324 strains of anaerobic bacteria. Apalcillin (a penicillin derivative) and cefpiramide (a semisynthetic cephalosporin) were compared with piperacillin, moxalactam, and cefoxitin. Organisms studied included the *Bacteroides fragilis* group, other *Bacteroides* species, fusobacteria, clostridia, nonsporeforming gram-positive rods, and anaerobic cocci. Piperacillin was found to be the most active overall, inhibiting 96% of the strains tested at its achievable level in serum (128 μg/ml). Apalcillin was comparable in activity to piperacillin, inhibiting 93% of anaerobes tested at this concentration. The other antibiotics inhibited ca. 80% of the strains at 32 μg/ml. In terms of activities against particular species, apalcillin was active against 75% of *B. fragilis* group strains and 97 to 100% of all other anaerobes. Cefpiramide inhibited 37% of *B. fragilis* group strains at 32 μg/ml and 68% at 64 μg/ml (a level that may be achievable with this drug). Cefpiramide inhibited 92% of all other anaerobes at 32 μg/ml and 95% at 64 μg/ml. The clostridia other than *Clostridium perfringens* were the most resistant (84% inhibited at 32 μg/ml and 95% inhibited at 64 μg/ml).

Many new beta-lactam antibiotics have been introduced in the last several years, many of which have limited activity against anaerobic bacteria (2, 3, 10, 13). Apalcillin is a naphthyridine derivative of ampicillin which has been reported to have levels of activity comparable to those of other broad-spectrum penicillin derivatives (e.g., azlocillin, mezlocillin, and piperacillin) against a wide range of organisms and particularly good activity against *Pseudomonas aeruginosa* (12). Cefpiramide (SM-1652) is a semisynthetic cephalosporin whose structure, antibacterial activity, and stability to β-lactamases have been described previously (4). Cefpiramide has been reported to have excellent activity against *P. aeruginosa* (4, 7, 9). The purpose of this study was to investigate the activities of these two new antimicrobial agents against a wide range of anaerobic bacteria, with particular attention to the *Bacteroides fragilis* group, which exhibits resistance to many of the newly developed antimicrobial agents.

MATERIALS AND METHODS

All bacterial strains were recent clinical isolates. Bacteria were identified by previously established procedures (5, 15). Apalcillin and cefpiramide were supplied by Wyeth Laboratories, Philadelphia, Pa. For comparison, three older antibiotics were studied at the same time. Cefoxitin was supplied by Merck Sharp & Dohme, West Point, Pa., moxalactam was supplied by Eli Lilly & Co., Indianapolis, Ind.; and piperacillin was supplied by Lederle Laboratories, Pearl River, N.Y. Antimicrobial powders were diluted as recommended by the manufacturers and prepared fresh before each experiment. MICs were determined by a blood agar plate dilution method previously described (15). The MIC was interpreted as the lowest concentration of each antimicrobial agent permitting no growth, two or fewer discrete colonies, or a barely visible haze.

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RESULTS AND DISCUSSION

The range of MICs and the minimum concentrations of cefpiramide and apalcillin required to inhibit 50 and 90% of the strains (MIC_{50} and MIC_{90}) tested, are listed in Table 1. MICs were determined for moxalactam, piperacillin, and cefoxitin as well; the data are shown only for cumulative percentages susceptible at the breakpoint (Table 2). Apalcillin was moderately active against some of the *B. fragilis* group (MIC_{50} = 32 μg/ml), but 12 of the 37 strains of *B. fragilis* were resistant even at 256 μg/ml. Thus, 25% (18 of 74) of *B. fragilis* group strains were resistant to the highest concentration of antibiotic tested. Others (12) have also reported similar results. Other *Bacteroides* species were more susceptible to apalcillin; only 2 of 75 strains were resistant at 256 μg/ml. *B. oralis, B. bivius, B. capillosus*, and *B. melaninogenicus* subsp. *melaninogenicus* were more resistant than the other non-*B. fragilis* group *Bacteroides* strains tested. All of the other anaerobes tested were susceptible to apalcillin at a concentration of 64 μg/ml or less.

Cefpiramide was also relatively weak in activity against the *B. fragilis* group; 22% (16 of 74) of strains of the *B. fragilis* group were resistant even at 128 μg/ml (10 of 37 *B. fragilis* strains, 3 of 18 *B. thetaiotaomicron* strains, 2 of 6 *B. distasonis* strains, and 1 of 5 *B. vulgatus* strains). Cefpiramide has been reported to be sensitive to the β-lactamases of the *B. fragilis* group, which are classified as cefuroxime-hydrolyzing enzymes (7). Of the other *Bacteroides* species, only *B. capillosus* exhibited significant resistance to cefpiramide. Cefpiramide was quite effective against the clostridia, excellent against the fusobacteria and cocci, and very active against gram-positive nonsporeforming rods, with only the *Eubacterium lentum* strains showing marked resistance (four of five strains were inhibited only at 128 μg/ml). These results correlate well with those reported previously (7, 9).

In comparing the antibacterial activities against *B. fragilis* of the five antibiotics tested at achievable levels in serum

TABLE 1. Susceptibility of anaerobic bacteria to apalcillin and cefpiramide

Microorganism (no. of isolates)	MIC (μg/ml)					
	Apalcillin			Cefpiramide		
	Range	50%	90%	Range	50%	90%
Actinomyces species[a] (6)	0.5–2	1	1	0.5–2	1	1
Bacteroides fragilis group (74)	2–>256	64	>256	8–>256	64	>256
B. fragilis (37)	16–>256	32	>256	8–>256	64	>256
B. thetaiotaomicron (18)	2–>256	64	>256	8–>256	64	>256
B. ovatus (8)	8–>256	32	64	16–128	32	128
B. distasonis (6)	16–>256	64	>256	128–>256	128	>256
B. vulgatus (5)	16–256	16	>256	16–256	32	256
Other *Bacteroides* species[b] (75)	0.062–256	4	64	0.062–>256	8	32
Clostridium difficile (32)	4–64	4	64	16–128	32	32
Clostridium perfringens (16)	0.062–1	0.25	0.5	0.25–4	2	2
Other *Clostridium* species[c] (24)	0.062–32	1	16	0.062–32	1	16
Eubacterium lentum (5)	2–32	16	32	1–128	128	128
Fusobacterium species[d] (25)	0.062–16	0.062	8	0.062–16	2	8
Peptococcus species[e] (31)	0.062–1	0.062	0.5	0.062–16	0.5	8
Peptostreptococcus species[f] (14)	0.062–16	0.25	8	0.062–8	1	8
Propionibacterium acnes (8)	0.25–2	1	2	0.25–5	0.5	0.5
Streptococcus intermedius (2)	0.5–64	0.5	64	0.5–16	0.5	16
Veillonella parvula (2)	32	32	32	4–8	4	8

[a] *Actinomyces* sp. (one isolate), *A. israelii* (one isolate), and *A. odontolyticus* (four isolates).

[b] *B. ruminicola* subsp. *brevis* (eight isolates), *B. ruminicola* subsp. *ruminicola* (three isolates), *B. melaninogenicus* subsp. *melaninogenicus* (thirteen isolates), *B. melaninogenicus* subsp. *intermedius* (sixteen isolates), *B. oralis* (five isolates), *B. bivius* (eight isolates), *B. disiens* (one isolate), *B. capillosus* (eight isolates), *B. ureolyticus* (six isolates), and *B. asaccharolyticus* (seven isolates).

[c] *C. cadaveris* (one isolate), *C. clostridiiforme* (two isolates), *C. innocuum* (three isolates), *C. ramosum* (four isolates), *C. septicum* (three isolates), *C. sordellii* (two isolates), *C. subterminale* (four isolates), and *C. tertium* (five isolates).

[d] *F. nucleatum* (nine isolates), *F. mortiferum* (three isolates), *F. gonidiaformans* (two isolates), *F. necrophorum* (three isolates), and *F. varium* (eight isolates).

[e] *P. asaccharolyticus* (twelve isolates), *P. magnus* (eleven isolates), and *P. prevotii* (eight isolates).

[f] *P. anaerobius* (10 isolates) and *P. micros* (four isolates).

(Table 2), we found that piperacillin was the most active against the *B. fragilis* group. Apalcillin was comparable to cefoxitin in its activity, although moxalactam and cefpiramide had poorer activities against this group. The percentage of inhibition of strains for cefpiramide was calculated for levels in serum of both 32 and 64 μg/ml, since the latter may be achievable with this drug.

None of the antimicrobial agents tested was very effective against the *B. fragilis* group, although some other investigators have found higher levels of activity with cefoxitin (1, 6, 8, 18), moxalactam (6, 14), and piperacillin (11) than was found in this study. Earlier studies from this laboratory yielded higher percentages of *B. fragilis* strains susceptible to piperacillin and cefoxitin (13). All five antimicrobial agents were approximately equally active against other *Bacteroides* species (90 to 97%).

All five drugs had excellent activities against *Clostridium perfringens*; apalcillin and piperacillin had excellent (100%) activities against clostridial species other than *C. perfringens* or *C. difficile*, whereas the other three antimicrobial agents

TABLE 2. Percentage of strains susceptible at the breakpoint[a]

Microorganism (no. of strains)	% of strains susceptible				
	Apalcillin (128)[b]	Piperacillin (64 [128][c])	Moxalactam (16 [32])	Cefoxitin (16 [32][c])	Cefpiramide[d] (32 [64])
Bacteroides fragilis group (74)	75	79 (85)	55 (63)	51 (76)	37 (68)
Other *Bacteroides* species (75)	97	97 (97)	79 (89)	93 (99)	93 (93)
Clostridium difficile (31)	100	100 (100)	0 (6)	0 (0)	87 (97)
Clostridium perfringens (16)	100	100 (100)	100 (100)	100 (100)	100 (100)
Other *Clostridium* species (24)	100	100 (100)	79 (88)	88 (88)	79 (92)
Fusobacterium species (25)	100	100 (100)	100 (100)	96 (100)	100 (100)
Gram-negative anaerobic cocci (2)	100	100 (100)	100 (100)	100 (100)	100 (100)
Gram-positive anaerobic cocci (47)	100	100 (100)	100 (100)	100 (100)	100 (100)
Gram-positive nonsporeforming bacilli (19)	100	100 (100)	79 (79)	100 (100)	79 (79)
Total	93	94 (96)	68 (77)	76 (83)	81 (88)

[a] Food and Drug Administration-approved breakpoints were listed on the package inserts for piperacillin, moxalactam, and cefoxitin and estimated for apalcillin and cefpiramide. Figures for moxalactam are for conventional and high-dosage (in parentheses) breakpoints.

[b] Breakpoints in micrograms per milliliter.

[c] Numbers in brackets are based on our conclusions concerning breakpoints at high dosages.

[d] The breakpoint for cefpiramide has not been determined; it is at least 32 μg/ml but may be 64 μg/ml.

showed good activities (80 to 88%) against this group. Apalcillin and piperacillin were the most active (100%) against *C. difficile*, cefpiramide had good (87%) activity, and moxalactam and cefoxitin were relatively inactive, as expected.

The results of this study correlate well with previous work done in our laboratory, with certain notable differences. In a previous study (13), Rolfe and Finegold found that cefoxitin inhibited 91% of anaerobic strains tested at its breakpoint of 32 μg/ml. Sutter and Finegold (16) found that 93% of anaerobes were inhibited at this concentration. In our study, cefoxitin inhibited 83% of anaerobic strains tested, with the notable difference occurring in the inhibition of *C. difficile*. When *C. difficile* is eliminated from the calculations, cefoxitin inhibited 92% of anaerobic strains in the present study. Moxalactam inhibited 79% of anaerobic strains overall, showing an excellent correlation with previous work (13); additionally, the correlation extends to the percentage of inhibition of individual species or groups.

The only notable exceptions in this study involve the fusobacteria. In a previous paper, Rolfe and Finegold (13) reported that 20% of strains of *Fusobacterium mortiferum* and *F. varium* were resistant to cefoxitin and moxalactam at their breakpoints, whereas in our study all of the fusobacteria were susceptible at these levels. The discrepancies are not due to differences in the random sampling of strains. Rather, the pattern of growth of several *Fusobacterium* strains exhibits confusing results in susceptibility patterns. Heavy growth is inhibited at the concentrations reported in this study; a haze (with visible, tiny, discrete colonies) persists at concentrations of up to 256 μg/ml with a few strains of *F. varium* and *F. mortiferum*. Studies are under way to clarify the nature of this growth, but preliminary observations indicate that this growth may represent cell wall-deficient forms of fusobacteria. This phenomenon has been reported earlier with fusobacteria (17).

These data indicate that although apalcillin and cefpiramide are of limited value against the *B. fragilis* group, their level of activity against most other anerobes compares favorably with that of the other antimicrobial agents tested.

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