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In Vitro Activity of Cefoperazone plus Sulbactam Compared with That of Other Antimicrobial Agents against Anaerobic Bacteria

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The activity of two cefoperazone-sulbactam combinations against anaerobic bacteria was tested and compared both with that of cefoperazone alone and with that of other commonly used antimicrobial agents. Imipenem was the most active of the tested agents, followed by chloramphenicol, metronidazole, and cefoperazone-sulbactam (90 to 100% of bacterial growth inhibited). Clindamycin and cefoxitin inhibited ~80%, cefoperazone inhibited 63%, and penicillin G inhibited 47% of the strains tested. The agents were variable in activity against the *Bacteroides fragilis* group, with percents susceptible as follows: cefoperazone-sulbactam, imipenem, metronidazole, and chloramphenicol, 99 to 100%; cefoxitin and clindamycin, ~80%; cefoperazone, 49%; and penicillin G, 15.5%.

β-Lactamase activity is generally considered the main mechanism of resistance of anaerobes to many of the broad-spectrum cephalosporins (10–12), and there is evidence that this activity is important in clinical resistance to certain β-lactam agents (6). Cefoperazone is a cephalosporin analog of piperacillin that is active against many gram-negative and gram-positive aerobic bacteria, but its in vitro activity against anaerobic bacteria is relatively poor. The addition of a β-lactamase inhibitor dramatically increases the effectiveness of many β-lactamase labile penicillins and cephalosporins against anaerobes (1, 2, 4, 5, 10). This study was undertaken to determine the effect of the addition of sulbactam to cefoperazone, with both a fixed ratio of cefoperazone to sulbactam and a fixed concentration of sulbactam, on the in vitro activity of cefoperazone against anaerobes and to compare this combination with other antimicrobial agents.

All bacteria used in this study were randomly selected recent clinical isolates from the Veterans Administration Wadsworth Medical Center, Los Angeles, Calif. Bacteria were identified by established procedures (7, 14). MICs were determined by a brucella blood agar plate dilution technique described previously (14), with an inoculum of 10^5 CFU. Plates were incubated in GasPak jars for 48 h at 37°C. MICs were defined as the lowest concentration of antimicrobial agent permitting either no growth, one discrete colony, or a barely visible haze. Reference strains of *Bacteroides fragilis* ATCC 25285 and *B. thetaiotaomicron* ATCC 29743 were used as controls in each test. Breakpoints were defined by the Food and Drug Administration-approved package inserts for high dosing of cefoperazone, cefoxitin, imipenem, clindamycin, and metronidazole and by our own estimates for penicillin G and chloramphenicol.

Clinical isolates were randomly screened in the broth disk procedure described by Kurzynski et al. (9) with disks containing cefoperazone alone and with disks containing both cefoperazone and sulbactam. Those isolates which were resistant to cefoperazone alone and susceptible to the cefoperazone-sulbactam combination were used in an in vitro susceptibility study using the agar dilution method (14) and a checkerboard titration dilution scheme. On the basis of our results, we decided to use two cefoperazone-sulbactam combinations in our study: (i) a fixed cefoperazone-sulbactam ratio of 2:1 and (ii) a fixed sulbactam concentration of 8 μg/ml plus twofold serial concentrations of cefoperazone. According to Dias et al. (4), levels of 16 μg of cefoperazone and 8 μg of sulbactam per ml are easily achievable in serum. Others have tested the cefoperazone-sulbactam combination both at a 2:1 ratio and at a fixed sulbactam concentration of 1, 2, 5, 8, or 10 μg/ml (1, 2, 4).

The results of the in vitro study with 310 strains of anaerobic bacteria are summarized in Table 1. Sulbactam alone was active against 87 to 90% of gram-negative anaerobic rods (the MIC was 32 μg/ml for almost 60% of the strains). Sulbactam alone was inactive against *Clostridium* species and relatively inactive against anaerobic cocci.

As noted in previous reports from our laboratory (16–18) and from others (3), it is crucial to differentiate between the species *B. fragilis* and the *B. fragilis* group. *B. fragilis* tends to be much more susceptible to many antimicrobial agents than the other members of the group. Therefore, we report the results for *B. fragilis* separately from those for the other members of the group. Ceftizoxime, cefotaxime, and penicillin G were included in this study, and the data were consistent with those previously published by our laboratory (17). Both cefoperazone-sulbactam combinations were active against almost the entire *B. fragilis* group (one strain of *B. uniformis* was resistant to the combination). Similarly, imipenem, metronidazole, and chloramphenicol were active against almost all of the strains tested. Cefoperazone without sulbactam was relatively inactive against both *B. fragilis* and the group as a whole. Cefoxitin and clindamycin were both active against ~90% of *B. fragilis* isolates and against ~80% of the *B. fragilis* group (~70% of non-*B. fragilis B. fragilis* group strains). All of the antimicrobial agents except penicillin G were active against ~84 to 100% of other *Bacteroides* species. *B. gracilis*, *B. denticola*, and *B. loescheii* accounted for the penicillin resistance among the non-*B. fragilis Bacteroides* species. The penicillin resistance of *B. gracilis*, an important pathogen in some deep-seated infections, has been noted previously in our laboratory (8). All of the antimicrobial agents were relatively active against the *Fusobacterium* species, although two strains of *Fusobacter-*

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TABLE 1. Comparative activity of antimicrobial agents against anaerobes

Organism (no. of strains tested)	Antimicrobial agent (breakpoint[a] [μg/ml])	MIC (μg/ml)		% Susceptible at breakpoint
		Range	90%[b]	
B. fragilis (82)	Sulbactam (32)	16–64	32	96.3
	Cefoperazone (32)	4–>256	>256	48.8
	Cefoperazone-sulbactam 2:1 (32)[c]	2–16[d]	16	100.0
	Cefoperazone-sulbactam (8 μg/ml)[e]	0.5–32[d]	8	100.0
	Cefoxitin (32)	8–128	32	91.5
	Penicillin G (16)	8–256	256	4.9
	Imipenem (8)	0.25–4	1	100.0
	Clindamycin (4)	0.25–256	4	91.5
	Metronidazole (16)	0.5–4	4	100.0
	Chloramphenicol (16)	4–16	16	100.0
B. fragilis group (excluding *B. fragilis*) (87[f])	Sulbactam (32)	4–256	64	84.7
	Cefoperazone (32)	0.5–256	128	49.4
	Cefoperazone-sulbactam (2:1)	0.5–128	32	98.8
	Cefoperazone-sulbactam (8 μg/ml)	0.5–64	32	98.8
	Cefoxitin (32)	2–128	64	70.6
	Penicillin G (16)	1–>256	>256	26.2
	Imipenem (8)	0.125–4	1	100.0
	Clindamycin (4)	0.0625–>256	256[g]	73.7
	Metronidazole (16)	0.25–>256	2	98.8
	Chloramphenicol (16)	0.5–16	16	100.0
Other *Bacteroides* spp.[h] (32)	Sulbactam (32)	1–128	64	87.5
	Cefoperazone (32)	0.5–>256	256	84.4
	Cefoperazone-sulbactam 2:1 (32)	0.5–256	32	96.7
	Cefoperazone-sulbactam (8 μg/ml)	0.25–>256	16	96.9
	Cefoxitin (32)	0.5–>256	64	87.5
	Penicillin G (16)	0.125–>256	>256	40.6
	Imipenem (8)	0.0625–2	1	100.0
	Clindamycin (4)	0.0625–32	4	93.8
	Metronidazole (16)	0.125–>256	2	90.6
	Chloramphenicol 916)	0.0625–8	4	100.0
Fusobacterium spp.[i] (37)	Sulbactam (32)	4–128	128	87.5
	Cefoperazone (32)	0.0625–>256	>256	88.2
	Cefoperazone-sulbactam 2:1 (32)	0.0625–>256	256	88.2
	Cefoperazone-sulbactam (8 μg/ml)	0.0625–>256	>256	88.2
	Cefoxitin (32)	0.0625–>256	256	88.2
	Penicillin G (16)	0.0625–2	2	100.0
	Imipenem (8)	0.0625–2	2	100.0
	Clindamycin (4)	0.0625–4	4	100.0
	Metronidazole (16)	0.0625–>256	1	94.1
	Chloramphenicol (16)	0.0625–4	4	100.0
C. difficile (38)	Sulbactam (32)	0–>256	>256	0.0
	Cefoperazone (32)	32–128	128	5.6
	Cefoperazone-sulbactam 2:1 (32)	1–256	256	5.6
	Cefoperazone-sulbactam (8 μg/ml)	64–256	256	0.0
	Cefoxitin (32)	128–>256	>256	0.0
	Penicillin G (16)	2–8	8	100.0
	Imipenem (8)	4–16	16	88.9
	Clindamycin (4)	4–>256	>256	5.6
	Metronidazole (16)	0.25–2	1	100.0
	Chloramphenicol (16)	4–64	64	72.2
C. perfringens (37)	Sulbactam (32)	128–256	128	0.0
	Cefoperazone (32)	1–8	8	100.0
	Cefoperazone-sulbactam (2:1)	4–8	8	100.0
	Cefoperazone-sulbactam (8 μg/ml)	0.5–8	8	100.0
	Cefoxitin (32)	2–4	4	100.0
	Penicillin G (16)	0.0625–1	1	100.0
	Imipenem (8)	0.0625–0.5	0.25	100.0
	Clindamycin (4)	0.0625–2	2	100.0
	Metronidazole (16)	0.0625–2	2	100.0
	Chloramphenicol (16)	4–16	8	100.0

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TABLE 1—*Continued*

Organism (no. of strains tested)	Antimicrobial agent (breakpoint[a] [μg/ml])	MIC (μg/ml)		% Susceptible at breakpoint
		Range	90%[b]	
Gram-positive nonsporeforming rods[j] (32)	Sulbactam (32)	0.5–256	128	78.3
	Cefoperazone (32)	0.125–256	128	84.4
	Cefoperazone-sulbactam (2:1)	0.5–256	32	84.4
	Cefoperazone-sulbactam (8 μg/ml)	0.25–>256	16	83.3
	Cefoxitin (32)	0.125–64	16	96.9
	Penicillin G (16)	0.0625–64	4	96.9
	Imipenem (8)	0.0625–2	0.5	100.0
	Clindamycin (4)	0.0625–128	16	66.7
	Metronidazole (16)	0.25–>256	>256	53.3
	Chloramphenicol (16)	0.5–32	8	96.7
Peptostreptococcus spp.[k] (25)	Sulbactam (32)	4–>256	256	56.0
	Cefoperazone (32)	0.125–8	4	100.0
	Cefoperazone-sulbactam (2:1)	0.5–32	8	100.0
	Cefoperazone-sulbactam (8 μg/ml)	0.5–16	8	100.0
	Cefoxitin (32)	0.0625–16	4	100.0
	Penicillin G (16)	0.0625–8	8	100.0
	Imipenem (8)	0.0625–1	0.12	100.0
	Clindamycin (4)	0.0625–>256	256	84.0
	Metronidazole (16)	0.125–>256	256	88.0
	Chloramphenicol (16)	1–64	8	96.0

[a] Breakpoints are those described in Food and Drug Administration package inserts for cefoperazone, cefoxitin, clindamycin, imipenem, and metronidazole. Breakpoint for chloramphenicol is our estimate.
[b] MIC for 90% of strains tested.
[c] The ratio of cefoperazone to sulbactam was 2:1 throughout the dilution series; the figure in parentheses is the breakpoint for cefoperazone.
[d] The MIC range, MIC for 90% of strains tested, and percent susceptible at breakpoint refer to concentrations of cefoperazone.
[e] A fixed concentration of 8 μg of sulbactam per ml was added throughout the dilution series.
[f] Includes 35 *B. thetaiotaomicron*, 13 *B. distasonis*, 14 *B. vulgatus*, 10 *B. ovatus*, 10 *B. uniformis*, and 5 *B. caccae* strains.
[g] This value is somewhat misleading: at 8 μg/ml, 85% of the strains are inhibited.
[h] Includes nine *B. denticola*, four *B. gracilis*, eight *B. intermedius*, two *B. loescheii*, and nine *B. melaninogenicus* strains.
[i] Includes eight *Fusobacterium necrophorum*, seven *F. nucleatum*, and two *F. varium* strains.
[j] Includes 11 *Actinomyces* species, 12 *Eubacterium* species, 5 *Lactobacillus* species, and 4 *Propionibacterium* species.
[k] Includes six *P. anaerobius*, seven *P. asaccharolyticus*, eight *P. magnus*, and four *P. prevotii* strains.

ium varium were resistant to cefoperazone as well as to the cefoperazone-sulbactam combinations. The only agents highly active against *Clostridium difficile* were penicillin G, imipenem, and metronidazole. Chloramphenicol was active against all but five strains, and the other agents were either almost or totally inactive. As expected, *C. perfringens* was inhibited by all of the antimicrobial agents except sulbactam. The gram-positive nonsporeforming rods were best inhibited by imipenem (100%), cefoxitin and penicillin G (96.9%), and chloramphenicol (96.7%). The other β-lactam agents tested were active against 78 to 90% of the strains, and the addition of sulbactam did not alter the efficacy of cefoperazone. Both metronidazole and clindamycin were relatively inactive (53.3 and 66.7%, respectively).

The anaerobic gram-positive cocci were best inhibited by the β-lactam agents (100%), except sulbactam, and by chloramphenicol (96%). Clindamycin and metronidazole were slightly less active (84 and 88%, respectively), and sulbactam was relatively inactive (56%). The metronidazole resistance is probably due to microaerophilic strains (13, 15).

This study confirms the reports of other investigators regarding activity of cefoperazone-sulbactam against the *B. fragilis* group (1, 2, 4, 5) and *Fusobacterium* species (1) and extends the data to include other species of anaerobic bacteria. Dias et al. (4) have reported similar results for *B. fragilis* and slightly higher resistance rates (e.g., 60 to 80% resistance) for non-*B. fragilis* *B. fragilis* group strains. However, they use a breakpoint of 16 μg/ml (the National Committee for Clinical Laboratory Standards-recommended breakpoint) rather than the 32-μg/ml breakpoint used in our laboratory (which is the breakpoint given in the Food and Drug Administration-approved package insert for high dosing). As pointed out previously, the clustering of MICs about the breakpoint for *B. fragilis* group isolates can cause significant variation in reported resistance rates if the breakpoint varies by even one dilution. Dias et al. reported synergy for cefoperazone and sulbactam even with β-lactamase-negative strains. Appelbaum et al. (1) found no synergy between β-lactams and three β-lactamase inhibitors (including sulbactam) against 30 *B. fragilis* group II isolates, while synergy against *B. fragilis* group I strains and other non-*B. fragilis* *B. fragilis* group strains was apparent. We did not differentiate between the two *B. fragilis* groups in this study. Values similar to ours for cefoperazone and a higher percentage of isolates susceptible to 16 μg of cefoxitin per ml than we saw at Wadsworth were also reported by Appelbaum et al. Fu and Neu state that the cefoperazone-clavulanic acid combination is synergistic against both constitutive β-lactamase producers and inducible β-lactamase strains of *B. fragilis* (5). Crosby and Gump report that they were not able to induce β-lactamase on β-lactamase-negative strains with subinhibitory concentrations of cefoperazone (2) (this was our experience with attempted induction with cefoxitin as well [H. Wexler, unpublished data]). Crosby and Gump suggest that a permeability barrier plays a role in the resistance of β-lactamase-negative strains, because the addition of EDTA (an agent known to increase permeability of gram-negative cell walls) more frequently enhanced the activity of cefoperazone against β-lactamase-negative isolates than against β-lactamase-positive isolates. Although the differences between dilution schemes make direct comparisons of data difficult, the results of Crosby and Gump are comparable to ours.

In summary, imipenem, chloramphenicol, and metronidazole remain the most active agents overall against anaerobes (93 to 99% inhibition of all strains tested); the cefoperazone-sulbactam combinations are nearly as active (91%). Cefoperazone-sulbactam was considerably more active than cefoxitin or clindamycin (both of which inhibited ~80%) and much more active than any of the other β-lactam agents except imipenem.

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