ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 1987, p. 267–273
0066-4804/87/020267-07$02.00/0

Vol. 31, No. 2

In Vitro Activity of CGP 31608, a New Penem

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Received 8 July 1986/Accepted 18 November 1986

The in vitro activity of CGP 31608, a semisynthetic penem derivative, was compared with that of Sch 34343, imipenem, cefoxitin, cefuroxime, and ceftazidime and other β-lactams, when appropriate, against 628 recent isolates and other β-lactam-resistant strains. The MICs of CGP 31608 against 90% of the members of the family *Enterobacteriaceae*, *Pseudomonas aeruginosa*, *Haemophilus influenzae*, *Neisseria* spp., *Bacteroides* spp., *Clostridium* spp., staphylococci, and *Streptococcus pneumoniae* were between 0.25 and 8 μg/ml. The susceptibility of β-lactamase-producing strains and known porin mutants of the *Enterobacteriaceae* suggests that CGP 31608 is resistant to many important β-lactamases (including the mutationally derepressed chromosomal enzymes) and is not excluded from the bacterial cell in strains expressing these known porin mutations. Generally, CGP 31608 was less active than imipenem, Sch 34343, and the cephalosporins, except against *Pseudomonas aeruginosa*. The activity of CGP 31608 against *Staphylococcus aureus* (including methicillin-resistant strains) was greater than that of the cephalosporins. The major target site in *Escherichia coli* K-12 for CGP 31608 was penicillin-binding protein 2. The serum protein binding of 5 μg of CGP 31608 per ml was 14%, and serum had little effect on activity.

The β-lactams continue to be the most widely used group of antibiotics, and many structural variations have been synthesized. The development of penem structures has, however, been less fruitful. Among those synthesized are Sch 29,482 (4), which is no longer under development, Sch 34343 (3), and FCE 22101 (13). CGP 31608 has the structure (5R, 6S, 8R)-2-aminomethyl-6-hydroxy-ethyl-2-penem-carboxylic acid (Fig. 1).

In this study we compared CGP 31608 with other β-lactams including penems and carbapenems against a wide range of recent isolates and strains whose mechanisms of resistance to certain of these agents are known. The affinity of CGP 31608 to the penicillin-binding proteins (PBPs) of *Escherichia coli* and *Bacteroides fragilis* were also studied.

MATERIALS AND METHODS

A total of 641 strains were studied, of which 628 were recent clinical isolates from this hospital (Table 1). Thirteen strains were well-characterized β-lactamase producers. The strains of *E. coli* DCO with altered expression of porin proteins, *ompR* (Omp C^-, Omp F^-) and *envZ* (Omp C^{++}, Omp F^-) mutants, were obtained from N. A. C. Curtis (2). The outer membrane protein profiles were checked both before and after the susceptibility testing.

The antimicrobial agents investigated were from the following sources: CGP 31608 from Ciba-Geigy, Basel, Switzerland; imipenem and cefoxitin from Merck Sharp & Dohme, Hoddesdon, England; cefuroxime and ceftazidime from Glaxo Group Research, Greenford, England; Sch 34343 from Schering Corp., Bloomfield, N.J.; ampicillin, carbenicillin, and penicillin from Beecham Research Laboratories, Brentford, England. Moxalactam (Eli Lilly, Swindon, England) was used in the study of the porin-deficient mutants.

Susceptibility testing. The susceptibility of the strains to the compounds was studied by a routine agar plate dilution method. The inocula were prepared as follows. For all strains except streptococci (including *Streptococcus pneumoniae*), *Neisseria* spp., *Haemophilus influenzae*, and anaerobes, the organisms were grown overnight in nutrient broth to yield a viable count of about 10^9 CFU/ml. Streptococci, *H. influenzae*, and *Neisseria* spp. were grown in brain heart infusion broth (Oxoid Ltd., Basingstoke, England) plus 1% supplement C (Difco, Surrey, England). *Bacteroides* spp. were grown in Wilkins-Chalgren broth (Oxoid Ltd.) plus 0.25% sodium succinate. Clostridia were grown in Wilkins-Chalgren broth supplemented with 1% Tween 80 (which had previously been shown to enhance growth). The viable counts were comparable in each broth.

The inocula were obtained by transferring 1 μl of an undiluted culture or a 1:100 dilution of the overnight culture to the surface of the antibiotic-containing agar by a multipoint inoculating device (Denley-Tech, Billinghurst, England). The final inocula on the plates were therefore 10^4 and 10^6 CFU.

The medium used for the agar dilution procedure was Iso-Sensitest agar (pH 7.2; Oxoid) and was supplemented as follows: 5% horse blood plus 1% supplement C to support growth of streptococci, *H. influenzae*, and *Neisseria* spp., for anaerobes Wilkins-Chalgren agar was used.

All plates were incubated in air at 37°C for 24 h, except for the following: the anaerobes were grown in an anaerobic cabinet in an atmosphere of 10% hydrogen, 10% carbon dioxide, and 80% nitrogen; the *H. influenzae* and *Neisseria* spp. were incubated in air enriched with 6% carbon dioxide. In addition, *Staphylococcus aureus* was also incubated in air

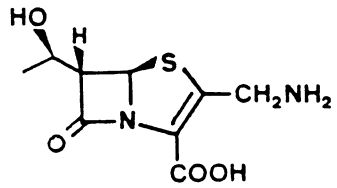

FIG. 1. Structure of CGP 31608.

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TABLE 1. The activity of CGP 31608 compared with other agents

Species (*n*)	Antibiotic	MIC (μg/ml)[a]		
		50%	90%	Range
Escherichia coli (50)	CGP 31608	4	4	0.5–8
	Imipenem	0.12	0.25	0.12–1
	Sch 34343	0.5	1	0.06–2
	Cefoxitin	4	16	0.25–128
	Cefuroxime	4	16	0.12–>128
	Ceftazidime	0.25	2	0.03–4
Klebsiella spp. (50)	CGP 31608	4	4	2–4
	Imipenem	0.5	1	0.25–1
	Sch 34343	0.25	0.5	0.12–4
	Cefoxitin	2	4	0.5–32
	Cefuroxime	2	32	0.5–>128
	Ceftazidime	0.12	0.5	0.03–1
Enterobacter spp. (3 *E. aerogenes*, 10 *E. cloacae*)	CGP 31608	4	8	1–8
	Imipenem	0.25	0.5	0.12–4
	Sch 34343	1	2	0.12–2
	Cefoxitin	64	>128	2–>128
	Cefuroxime	16	>128	2–>128
	Ceftazidime	0.25	32	0.06–64
Proteus mirabilis (51)	CGP 31608	2	4	1–8
	Imipenem	1	2	0.12–4
	Sch 34343	0.5	1	0.25–2
	Cefoxitin	2	4	1–32
	Cefuroxime	1	16	0.5–>128
	Ceftazidime	0.03	0.12	0.03–0.5
Proteus vulgaris (19)	CGP 31608	2	4	1–16
	Imipenem	2	4	0.25–8
	Sch 34343	1	1	0.25–2
	Cefoxitin	2	4	2–8
	Cefuroxime	16	128	1–>128
	Ceftazidime	0.03	0.06	0.03–0.06
Morganella morganii (25)	CGP 31608	4	8	2–8
	Imipenem	0.5	2	0.12–4
	Sch 34343	1	2	0.5–4
	Cefoxitin	8	8	4–16
	Cefuroxime	16	64	0.25–128
	Ceftazidime	0.03	4	0.015–8
Citrobacter freundii (10)	CGP 31608	4	4	2–4
	Imipenem	0.25	0.25	0.12–0.25
	Sch 34343	0.5	1	0.25–1
	Cefoxitin	64	>128	2–>128
	Cefuroxime	4	>128	2–>128
	Ceftazidime	4	128	0.12–>128
Serratia spp. (17 *S. marcescens*, 3 *S. liquefaciens*)	CGP 31608	8	8	8
	Imipenem	0.5	0.5	0.25–0.5
	Sch 34343	2	4	1–4
	Cefoxitin	16	16	4–32
	Cefuroxime	128	>128	16–>128
	Ceftazidime	0.12	0.25	0.03–0.25
Salmonella spp. (15)	CGP 31608	2	4	2–4
	Imipenem	0.12	0.5	0.12–1
	Sch 34343	0.25	0.5	0.25–0.5
	Cefoxitin	2	4	2–4
	Cefuroxime	4	4	4
	Ceftazidime	0.25	0.5	0.12–0.5
Shigella spp. (8 *S. sonnei*, 3 *S. flexneri*, 2 *S. boydii*)	CGP 31608	4	4	1–4
	Imipenem	0.12	0.25	0.12–0.25
	Sch 34343	0.25	0.5	0.25–0.5
	Cefoxitin	2	32	2–32
	Cefuroxime	2	32	2–32
	Ceftazidime	0.12	4	0.12–4
Providencia spp. (8 *P. stuartii*, 2 *P. rettgeri*)	CGP 31608	1	8	1–8
	Imipenem	0.5	4	0.12–4
	Sch 34343	0.5	2	0.25–2
	Cefoxitin	4	32	2–64
	Cefuroxime	2	16	2–64
	Ceftazidime	0.25	0.5	0.12–8

Continued

TABLE 1—*Continued*

Species (*n*)	Antibiotic	MIC (μg/ml)[a]		
		50%	90%	Range
Pseudomonas aeruginosa (50)	CGP 31608	1	2	0.5–8
	Imipenem	2	4	0.25–8
	Sch 34343	128	>128	0.5–>128
	Cefoxitin	>128	>128	32–>128
	Cefuroxime	>128	>128	64–>128
	Ceftazidime	1	16	0.25–>128
	Carbenicillin	64	>128	32–>128
Acinetobacter anitratus (17)	CGP 31608	2	2	1–8
	Imipenem	0.12	0.25	0.12–1
	Sch 34343	2	8	0.25–8
	Cefoxitin	32	64	4–128
	Cefuroxime	32	64	2–128
	Ceftazidime	4	8	0.25–8
Haemophilus influenzae (35; including 11 β-lactamase positive)	CGP 31608	0.5	2	0.25–4
	Imipenem	0.5	2	0.25–4
	Sch 34343	0.5	2	0.25–2
	Cefoxitin	2	4	1–8
	Cefuroxime	0.5	16	0.5–16
	Ceftazidime	0.06	0.5	0.03–1
	Ampicillin	1	8	0.25–32
Neisseria gonorrhoeae (31; including 6 β-lactamase positive)	CGP 31608	0.5	2	0.25–2
	Imipenem	0.015	0.03	0.004–0.06
	Sch 34343	0.06	0.06	0.06–0.12
	Cefoxitin	0.25	0.5	0.12–1
	Cefuroxime	0.015	0.12	0.004–0.12
	Ceftazidime	0.015	0.03	0.004–0.03
	Penicillin	0.06	16	0.004–64
Bacteroides spp. (23 *B. fragilis*, 5 *B. thetaiotaomicron*, 1 *B. vulgatus*, 1 *B. distasonis*, 3 *B. ovatus*, 2 *B. asaccharolyticus*, 2 *B. uniformis*, 1 *B. bividus*)	CGP 31608	1	4	0.5–4
	Imipenem	0.25	0.5	0.06-4
	Sch 34343	0.12	0.5	0.03–16
	Cefoxitin	4	32	2–128
	Cefuroxime	64	>128	4–>128
	Ceftazidime	128	>128	4–>128
Clostridium spp. (15; including 3 *C. difficile*)	CGP 31608	2	8	0.5–32
	Imipenem	0.03	1	0.015–1
	Sch 34343	0.25	1	0.06–2
	Cefoxitin	1	8	0.25–16
	Cefuroxime	2	32	0.12–64
	Ceftazidime	2	128	0.5–>128
Staphylococcus aureus (50; including methicillin-resistant strains)	CGP 31608	0.12	0.25	0.06–0.5
	Imipenem	0.015	0.03	0.008–0.12
	Sch 34343	0.06	0.12	0.03–0.5
	Cefoxitin	2	4	0.5–8
	Cefuroxime	0.5	1	0.25–32
	Ceftazidime	8	8	4–32
Staphylococcus epidermidis (20)	CGP 31608	0.12	0.25	0.06–0.25
	Imipenem	0.015	0.03	0.004–0.12
	Sch 34343	0.06	0.12	0.03–0.25
	Cefoxitin	1	1	0.5–4
	Cefuroxime	0.25	0.25	0.25–2
	Ceftazidime	4	4	4–8
Staphylococcus saprophyticus (25)	CGP 31608	0.12	0.25	0.12–0.25
	Imipenem	0.03	0.03	0.015–0.06
	Sch 34343	0.25	0.25	0.06–0.5
	Cefoxitin	1	2	0.5–2
	Cefuroxime	1	2	0.25–2
	Ceftazidime	4	16	4–32
Streptococcus faecalis (20)	CGP 31608	16	16	16
	Imipenem	0.5	1	0.25–1
	Sch 34343	4	4	2–4
	Cefoxitin	>128	>128	1–>128
	Cefuroxime	64	128	64–>128
	Ceftazidime	128	>128	64–>128
	Penicillin	2	4	2–4
Streptococcus pneumoniae (28)	CGP 31608	0.25	0.25	0.12–2
	Imipenem	0.008	0.008	0.004–0.06
	Sch 34343	0.03	0.03	0.03–0.12
	Cefoxitin	1	1	0.5–16

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TABLE 1—*Continued*

Species (*n*)	Antibiotic	MIC (μg/ml)[a]		
		50%	90%	Range
	Cefuroxime	0.015	0.015	0.015–2
	Ceftazidime	0.12	0.12	0.06–2
	Penicillin	0.015	0.03	0.008–0.5
Streptococcus pyogenes Lancefield group A (10)	CGP 31608	0.5	0.5	0.25–0.5
	Imipenem	0.004	0.008	0.004–0.008
	Sch 34343	0.03	0.03	0.03–0.06
	Cefoxitin	0.05	0.5	0.25–0.5
	Cefuroxime	0.008	0.015	0.008–0.015
	Ceftazidime	0.12	0.12	0.06–0.12
	Penicillin	0.015	0.03	0.008–0.03
Streptococcus agalactiae Lancefield group B (10)	CGP 31608	0.5	0.5	0.5
	Imipenem	0.015	0.015	0.015
	Sch 34343	0.06	0.06	0.06
	Cefoxitin	2	2	2
	Cefuroxime	0.03	0.03	0.03
	Ceftazidime	0.25	0.5	0.25–0.5
	Penicillin	0.06	0.06	0.06

[a] 50% and 90%, MIC for 50 and 90% of strains, respectively.

at 30°C with 5% sodium chloride added to the medium. The MIC of the antibiotic was defined as that concentration (in micrograms per milliliter of agar) at which to no more than two colonies were detected. In the case of the higher inoculum, a slight haze of growth was ignored.

The effect of human serum on the MIC and MBC of CGP 31608 was studied with nine strains (two each of *Klebsiella* spp., *Proteus mirabilis*, *E. coli*, and *Pseudomonas aeruginosa* and one strain of *Staphylococcus aureus*) by a method based on that Pearson et al. (7); the bactericidal endpoint was 99.9% lethality. An overnight broth culture of these organisms was inoculated into 1 ml of Iso-Sensitest broth with 0, 20, and 70% human serum and decreasing concentrations of the antimicrobial agent.

The protein binding of CGP 31608 was estimated in quadruplicate in human serum by an ultrafiltration technique with an Amicon Corp. (Lexington, Mass.) Centriflo cone with an exclusion limit of 50,000 daltons. The concentrations of CGP 31608 used were 5 and 100 μg/ml. The ultrafiltrate (after pH adjustment to precentrifugation values with CO_2 gas) was assayed by a microbiological method against standards prepared in phosphate buffer at pH 6.5 (the pH of the ultrafiltrate). The indicator organism was *E. coli* Sch 12655 (from Squibb Research, Princeton, N.J.), and the medium was Oxoid antibiotic no. 1.

The PBP affinity and morphological response to CGP 31608 were studied in *E. coli* KL-12 DCO and *B. fragilis* NCTC 9343. Bacterial envelopes were prepared, and the competition with [^{14}C]benzylpenicillin for the PBPs was determined as described previously (1, 8). The effect of various concentrations of CGP 31608 on the morphology of both test organisms in broth cultures was monitored by differential interference microscopy; results were recorded photographically.

RESULTS

The results obtained from 625 recent isolates tested at an inoculum of 10^4 CFU are summarized in Table 1. Although CGP 31608 was not as active as some of the other agents against members of the *Enterobacteriaceae*, it was noteworthy that all the MICs for 90% of the strains tested were between 4 and 8 μg/ml and all but one strain (a *Proteus vulgaris*; MIC, 16 μg/ml) were susceptible to 8 μg/ml. With the exception of the *Proteus* spp., CGP 31608 was 4- to 16-fold less active than imipenem and Sch 34343. Generally, CGP 31608 displayed activity against the *Enterobacteriaceae* that was similar to that of cefoxitin and was 4- to 16-fold less active than ceftazidime, with the exception of the *Enterobacter* spp., *Citrobacter freundii*, and some strains of *Serratia marcescens* and *Providencia* spp., when CGP 31608 was the more active. Table 2 shows the activity of CGP 31608 and ceftazidime against 10 characterized β-lactamase-producing strains. Against strains expressing Richmond and Sykes (10) group I β-lactamase, neither compound showed reduced activity for the *ampC* strain, but ceftazidime did appear to be less active against one strain of *Enterobacter cloacae* (1051E) possessing the P99 enzyme and against a derepressed mutant (K299); this was not observed in the case of CGP 31608. Those strains containing Richmond and Sykes group III, IV, and V enzymes did not show any decreased susceptibility to ceftazidime or CGP 31608. Also studied was the activity of CGP 31608 and the β-lactams against two outer membrane porin mutants of a strain of *E. coli*. Alterations in the expression of OmpC and OmpF porins showed no significant change in the susceptibility to CGP 31608.

Against *P. aeruginosa*, the activity of CGP 31608 was comparable to that of imipenem, for which no strain was susceptible to >8 μg/ml, in comparison to ceftazidime, for which five strains were susceptible to ≥32 μg/ml. Sch 34343 had no significant activity against *P. aeruginosa*. Included in the 50 *P. aeruginosa* strains studied were strains expressing β-lactamases, the PSE-3, Dalgleish, TEM-1, and a chromosomal cephalosporinase (pI 8.7); all these strains were susceptible to ≤1 μg of CGP 31608 per ml. Three recent clinical isolates were susceptible to 8 μg of CGP 31608 per ml, and these strains tended to be less susceptible to ceftazidime (MICs, 32, >128, and 4 μg/ml) but not to imipenem (MICs, 4, 1, and 2 μg/ml, respectively).

Strains of *H. influenzae* (including 11 β-lactamase producers) were equally susceptible to CGP 31608, Sch 34343, and imipenem, but ceftazidime was about fourfold more active. Also included were six strains of non-β-lactamase-mediated ampicillin-resistant *H. influenzae* which were less susceptible to CGP 31608 (three with MICs of 4 μg/ml); these three

TABLE 2. Activity of CGP 31608 and ceftazidime on members of the *Enterobacteriaceae* with known mechanisms of resistance

Organism	Mechanism of resistance	CGP 31608 MIC		Ceftazidime MIC	
		10^4 CFU	10^6 CFU	10^4 CFU	10^6 CFU
Escherichia coli 1541E	β-Lactamase group I[a] (*ampC*)	4	4	4	4
Enterobacter cloacae 1051E	β-Lactamase group I ($P99^+$)	4	4	32	32
Enterobacter cloacae 1321E	β-Lactamase group I (P99)	1	2	1	1
Citrobacter freundii K293	β-Lactamase group I (DRM)[b]	4	4	128	128
Enterobacter cloacae K299	β-Lactamase group I (DRM)	4	4	32	64
Escherichia coli I153	β-Lactamase group III (Tem-1)	4	4	0.15	0.15
Escherichia coli I147	β-Lactamase group III (Tem-2)	4	4	0.5	0.5
"*Klebsiella aerogenes*" (H130)	β-Lactamase group III (SHV-1)	4	4	0.12	0.25
"*Klebsiella aerogenes*" (H132)	β-Lactamase group IV (K-1)	4	4	0.06	0.12
Escherichia coli I150	β-Lactamase group V (Oxa-1)	4	4	0.25	0.25
Escherichia coli I162	Omp C^{++} Omp F^- (*envZ*)	4	4	2[c]	2[c]
Escherichia coli I169	Omp C^- Omp F^- (*ompR*)	4	4	16[d]	16[d]

[a] Richmond and Sykes β-lactamase classification (10).
[b] DRM, Derepressed mutant.
[c] Moxalactam MIC.
[d] Cefoxitin MIC.

strains were also relatively less susceptible to Sch 34343 (MICs, 2, 2, and 1 μg/ml) and imipenem (MICs, 4, 2, and 1 μg/ml, respectively). Strains of *Neisseria gonorrhoeae* (including six β-lactamase producers) were susceptible to ≤2 μg of CGP 31608 per ml but 16- to 64-fold more susceptible to imipenem, ceftazidime, Sch 34343, and cefuroxime. Six strains of *Neisseria meningitidis* were tested (data not shown); all were susceptible to 1 to 2 μg of CGP 31608 per ml.

Against *Bacteroides* spp., CGP 31608 showed good activity; all strains were susceptible to ≤4 μg/ml. Six cefoxitin-resistant (MIC, ≥32 μg/ml) strains (two *Bacteroides fragilis*, two *Bacteroides thetaiotaomicron*, one *Bacteroides ovatus*, and one *Bacteroides distasonis*) were all susceptible to ≤4 μg of CGP 31608 per ml. One of the cefoxitin-resistant *B. fragilis* strains (MIC, 32 μg/ml) was less susceptible to imipenem (MIC, 4 μg/ml) and Sch 34343 (MIC, 16 μg/ml). Six strains of *Clostridium perfringens* were tested and all were susceptible to ≤2 μg/ml of CGP 31608. The three strains of *Clostridium difficile* were less susceptible to CGP 31608 (MICs, 8, 8 and 16 μg/ml).

S. aureus, *Staphylococcus epidermidis*, and *Staphylococcus saprophyticus* were all highly susceptible to CGP 31608 (MICs for 90% of the strains, 0.25 μg/ml) and Sch 34343 (MICs for 90% of the strains, 0.12 to 0.25 μg/ml) and about fourfold more susceptible to imipenem, but CGP 31608 and Sch 34343 were eightfold more active than cefoxitin or cefuroxime (with the exception that the latter was more active against *S. epidermidis*). The seven methicillin-resistant (MIC, ≥8 μg/ml) strains of *S. aureus* tended to be more resistant to CGP 31608 with the MIC raised two- to fourfold compared with those for the methicillin-susceptible strains. When 5% sodium chloride was added to the medium and incubation was at 30°C there was a further, on average, twofold increase in the MIC of CGP 31608 for the methicillin-resistant strains, to 0.25 to 0.5 μg/ml. Penicillin-resistant, methicillin-susceptible strains were as susceptible to CGP 31608 as the penicillin-susceptible strains. CGP 31608 was fourfold less active than benzylpenicillin against *Streptococcus faecalis* but displayed moderate activity against the *S. pneumoniae* and Lancefield group A and B streptococci. Against these last three pathogens, CGP 31608 was broadly similar to ceftazidime.

TABLE 3. Effect of increased percentage of human serum on the MICs and MBCs of CGP 31608[a]

Organism (*n*)	0% Serum		20% Serum		70% Serum	
	MIC	MBC	MIC	MBC	MIC	MBC
Escherichia coli						
1	4	8	4	16	4	16
2	4	32	4	32	8	16
Klebsiella pneumoniae						
1	4	4	4	8	4	32
2	4	8	4	16	4	8
Proteus mirabilis						
1	8	32	4	32	4	16
2	4	32	4	64	4	16
Pseudomonas aeruginosa						
1	8	64	16	128	16	64
2	2	16	2	16	4	32
Staphylococcus aureus	0.12	0.12	0.12	1	0.5	1

[a] MICs and MBCs are expressed as micrograms per milliliter of serum.

An increase in inoculum from 10^4 to 10^6 CFU had remarkably little effect on the activity of CGP 31608; at most there was a doubling of the MIC.

The mean protein binding of CGP 31608 at 5 μg/ml was 14% (range, 12 to 16%), and at 100 μg/ml it was 9% (range, 6 to 11%). In Table 3 the effect of serum on the MICs and MBCs is shown. There was usually a two- to 4-fold difference between the MIC and MBC, with the exception of the *P. mirabilis* and *P. aeruginosa*, for which there was an 8- to 16-fold difference. Generally, serum had little effect on the MIC or MBC.

Densitometer evaluation of the PBP fluorographs indicated that the primary target in *E. coli* DCO for CGP 31608 was PBP 2 (I_{50}, 0.7 μg/ml). With increasing concentrations of CGP 31608 the I_{50}s for PBP 1A and PBP 4 were 6 and 50 μg/ml, respectively. PBPs 1B, 3, 5, and 6 had an I_{50} of >256 μg/ml. The morphological response of *E. coli* exposed to various concentrations of CGP 31608 (0.5 to 256 μg/ml) showed cell rounding at 2 h. For *B. fragilis* NCTC 9343, PBP 3 was primarily inhibited; this protein was comparable to PBP 2 in *E. coli* (8).

DISCUSSION

The major difference between CGP 31608 and two previously studied penems, FCE 22101 and Sch 34343, is the considerable activity of the new compound against *P. aeru-*

ginosa; FCE 22101 and Sch 34343 have no activity against this pathogen (3, 11). Against other pathogens, including members of the *Enterobacteriaceae*, staphylococci, and anaerobes, CGP 31608 is generally one-fourth as active as the other penems. This in general is the finding in other preliminary studies (G. M. Eliopoulos, C. Wennerstern, E. Reiszner, and R. C. Moellering, Program Abstr. 26th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 633, 1986; D. S. Reeves, M. J. Bywater, and H. A. Holt, 26th ICAAC, abstr. no. 636, 1986). A noteworthy feature is the narrow range of susceptibility of members of the *Enterobacteriaceae* to CGP 31608 in contrast to the marked range of susceptibility to newer cephalosporins (e.g., ceftazidime; MICs, 0.03 to >128 μg/ml). CGP 31608 could therefore be said to possess a predictable degree of antimicrobial activity against a wide range of pathogens.

CGP 31608 also appears to be active against organisms expressing a wide range of β-lactamases including those of the *Bacteroides* spp. and those found in some strains of *H. influenzae* and *N. gonorrhoeae*. In the studies on the characterized β-lactamase-producing strains it is important to note that CGP 31608 may be more resistant to hydrolysis by the Richmond and Sykes (10) group I enzyme than the reference agent, ceftazidime. In particular the two derepressed high β-lactamase producing strains (*E. cloacae* K293 and K299) were markedly more susceptible to CGP 31608 than to ceftazidime. Since such strains are now being reported with increasing frequency from patients being treated with the more potent cephalosporins, it is important to study this point further by detailed hydrolysis studies. The preliminary information available from this investigation would suggest that, like imipenem (5), CGP 31608 will be active against such strains. However, it is probable that CGP 31608 is susceptible to β-lactamases which are zinc metaloenzymes, such as those carried by *Pseudomonas maltophilia*. We tested two such strains (CGP 31608 MICs, 32 μg/ml; data not shown), and others have reported resistance (H. C. Neu, N. M. Neu, P. Labthavikul, and N. X. Chin, 26th ICAAC, abstr. no. 634, 1986). Those mutations to the outer membrane protein genes (*ompR* and *envZ*) of *E. coli* which affect the permeability of some β-lactams (such as cefoxitin and moxalactam) into the bacterial cell (6) do not appear to affect CGP 31608. The significance of this in clinical practice is not well understood.

CGP 31608 together with the other penems, including FCE 22101 (11) and imipenem, shares high activity against staphylococci and is considerably more active than the so-called third-generation (broad-spectrum) cephalosporins, including ceftazidime. This high activity also included the methicillin-resistant strains of *S. aureus*, which were only two- to fourfold less susceptible than the methicillin-susceptible strains. These data are in agreement with those of some workers (Reeves et al., 26th ICAAC, abstr. no. 636, 1986) but disagree with those of others (H. Chambers, M. Sachdeva, F. Stella, C. Hackbarth, and M. A. Sande, 26th ICAAC, abstr. no. 632, 1986). The significance of this is difficult to assess but might be worthy of detailed in vivo study.

The primary target in *E. coli* and *B. fragilis* is the PBP involved in the maintenance of cell shape (PBP 2 and PBP 3 for each species, respectively). These data are similar to those of Mett et al. (H. Mett, P. Schacher, P. Schneider, and O. Zak, 26th ICAAC, abstr. no. 639, 1986), except that in this study inhibition of the nonlethal target PBP 4 in *E. coli* DCO was at a concentration well above the CGP 31608 MIC for this strain. The morphological response of each species growing in the presence of CGP 31608 was also consistent with the primary target being PBP 2 in *E. coli*. *B. fragilis* exhibited multiple morphological responses due to inhibition of more than one major PBP; this compares well with data already obtained for imipenem and mecillinam (8). The lytic effect of CGP 31608 was examined with a spectrophotometer at 675 nm (data not shown); some inhibition of growth was observed, but very little lysis was seen even at CGP 31608 concentrations 20 times the MIC. The killing kinetics of CGP 31608 (data not shown) with low inocula (10^4 CFU) of midlogarithmic-phase cells demonstrated rapid kill, with the rate of kill decreasing with an increase in cell numbers. However, even at an initial inoculum of 10^7 CFU of *B. fragilis* a 2-$\log_{10}$ drop in cell numbers was achieved at 1 μg/ml after 6 h of incubation, unlike most broad-spectrum cephalosporins. The two- to eightfold difference between the MIC and MBC is probably due to the target site inhibition, since inhibition of PBP 2 is primarily a static response. This compares well with the data for mecillinam (9); however imipenem, which inhibits PBPs la and lb at concentrations close to the MIC, does not show this difference between the MIC and MBC (12). Serum has little effect on the activity of the compound, as might be expected from the extremely low protein binding, a property which might be expected to contribute to good tissue penetration of this agent (13).

CGP 31608 therefore appears to be a promising agent for the treatment of a wide range of pathogens, including all the important members of the *Enterobacteriaceae*, *P. aeruginosa*, and anaerobes and the majority of gram-positive cocci. These properties should encourage its clinical study.

ACKNOWLEDGMENT

We thank O. Zak for his valued advice and support.

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