ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 1980, p. 939-943
0066-4804/80/12-0939/05$02.00/0
Vol. 18, No. 6

CI-867, a New Semisynthetic Penicillin: In Vitro Studies

SUSANNE S. WEAVER AND GERALD P. BODEY*

Department of Developmental Therapeutics, The University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, Houston, Texas 77030

CI-867, a new semisynthetic penicillin, has exhibited broad-spectrum activity in vitro against gram-positive cocci, except penicillin G-resistant *Staphylococcus aureus*, and against gram-negative bacilli. It was especially active against *Pseudomonas aeruginosa* and as active as mezlocillin and piperacillin against *Klebsiella pneumoniae*. CI-867 was bactericidal against most organisms. Its activity was greatly reduced when the inoculum was increased from 10^5 to 10^7 organisms per ml.

The past decade has witnessed the introduction of many new derivatives of penicillins with structural modifications which have increased their spectrum of activity (1, 3, 4). CI-867, $C_{32}H_{35}N_5O_{11}S_2Na_1$ (Fig. 1), a new semisynthetic penicillin prepared from amoxicillin, is the most recent of these derivatives (7, 10). This report presents the results of in vitro studies with this drug which indicate its broad spectrum of activity and its superior activity against *Pseudomonas aeruginosa* in comparison studies with carbenicillin, ticarcillin, mezlocillin, azlocillin, piperacillin, and ampicillin.

MATERIALS AND METHODS

A total of 631 clinical isolates, 471 gram-negative bacilli and 160 gram-positive cocci, were examined in this study. All gram-negative bacilli, except a few isolates of indole-positive *Proteus* spp., were cultured from blood specimens obtained from cancer patients at this institution. Isolates of gram-positive cocci and a few isolates of indole-positive *Proteus* spp. were obtained from cultures of various body sites of hospitalized patients, some of whom did not have cancer.

Minimal inhibitory concentrations and minimal bactericidal concentrations were determined in duplicate by procedures described previously (11). Except for tests on isolates of *Streptococcus pyogenes* and *Streptococcus pneumoniae*, which were made in tryptose-phosphate broth (Difco Laboratories), Mueller-Hinton broth served as the test medium. An inoculum of 10^5 cells per ml was used for all organisms except *S. pyogenes* and *S. pneumoniae*, for which 10^6 cells per ml was used. Two groups of *Staphylococcus aureus* isolates, one penicillin G susceptible and the other penicillin G resistant, were studied. The penicillin G minimal inhibitory concentrations for isolates in the susceptible group were $\leq$0.10 μg/ml, and those for isolates in the resistant group were $\geq$25 μg/ml. None of the isolates for which minimal inhibitory concentrations were in an intermediate range (0.20 to 12.5 μg/ml) was used for testing.

CI-867 was supplied by Warner-Lambert/Parke-Davis, Ann Arbor, Mich. Carbenicillin, ticarcillin, and ampicillin were from Beecham Laboratories, Bristol, Tenn. Piperacillin was from Lederle Laboratories, Pearl River, N.Y., and mezlocillin and azlocillin were from Delbay Research Corp., Florham, N.J.

RESULTS

The in vitro activity of CI-867 against 631 clinical isolates is summarized in Table 1. A concentration of 3.12 μg/ml inhibited a majority of all isolates of gram-positive cocci except penicillin G-resistant *S. aureus*. A concentration of 25 μg/ml inhibited only 11% and a concentration of 200 μg/ml inhibited only 58% of these isolates. CI-867 was active against a majority of isolates of *Enterobacteriaceae*. A concentration of 25 μg/ml inhibited 75% of isolates of *Escherichia coli*, *Enterobacter* spp., and *Serratia marcescens*, and 50 μg/ml inhibited 75% of isolates of *Klebsiella pneumoniae* and indole-positive *Proteus* spp. CI-867 was especially active against *P. aeruginosa*. A concentration of 12.5 μg/ml inhibited 90% of these isolates. Twenty percent of isolates of *E. coli*, 16% of *K. pneumoniae*, 8% of *Enterobacter* spp., 6% of *S. marcescens*, and 24% of *Proteus* spp. (indole positive) were resistant to CI-867 even at a concentration of 200 μg/ml. CI-867 was bactericidal at concentrations expected to be achieved clinically against a majority of isolates of all organisms except *S. marcescens* and enterococci. A concentration of 100 μg/ml inhibited 88% of isolates of *S. marcescens* and all isolates of enterococci but was bactericidal against only 34 and 22%, respectively.

The comparative activity of CI-867 in Mueller-Hinton broth, Trypticase soy broth (BBL Microbiology Systems), brain heart infusion broth, and nutrient broth and its comparative activity in Mueller-Hinton broth at pH 6.4, 7.2, and 8.0 were determined for 30 isolates each of *E. coli*, *K. pneumoniae*, and *P. aeruginosa*. Neither the type of broth nor the pH of Mueller-Hinton broth had an appreciable effect on the activity of CI-867.

The effect of a change in inoculum from 10^5 to 10^7 cells per ml on the activity of CI-867 against these same 30 isolates is shown in Fig. 2. Whereas concentrations of 1.56, 6.25, and 50 μg/ml inhibited all isolates of *E. coli*, *P. aeruginosa*, and *K. pneumoniae*, respectively, using an inoculum of 10^5 cells per ml, none of these isolates was inhibited by 200 μg of CI-867 per ml using an inoculum of 10^7 cells per ml.

The activity of CI-867 in comparison with those of carbenicillin, ticarcillin, mezlocillin, azlocillin, piperacillin, and ampicillin is shown in Table 2. CI-867 was the most active antibiotic against isolates of *P. aeruginosa*. It was twofold more active than piperacillin, the next most active antibiotic, and fourfold more active than azlocillin against 50% of these isolates. CI-867 was slightly less active than mezlocillin, piperacillin, or ticarcillin against isolates of *E. coli*, although all antibiotics inhibited 75% at achievable concentrations. CI-867 was as active as mezlocillin and piperacillin against isolates of *K.*

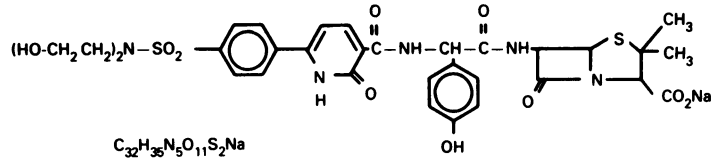

FIG. 1. *Structural formula of CI-867.*

TABLE 1. *In vitro activity of CI-867 against gram-negative bacilli and gram-positive cocci*

Organism (no. of isolates tested)	Activity tested[a]	Concnt (μg/ml) which inhibited the following percentage of isolates:		
		50%	75%	90%
Escherichia coli (100)	MIC	1.56	25	>200
	MBC	12.5	200	>200
Klebsiella pneumoniae (100)	MIC	25	50	>200
	MBC	25	100	>200
Pseudomonas aeruginosa (100)	MIC	3.12–6.25	6.25	12.5
	MBC	6.25	12.5	>200
Enterobacter spp. (50)	MIC	3.12	25	200
	MBC	12.5	50	>200
Serratia marcescens (50)	MIC	6.25–12.5	25	200
	MBC	200	>200	>200
Proteus mirabilis (50)	MIC	3.12	3.12	6.25
	MBC	3.12	3.12	6.25
Proteus spp. (21) (indole positive)	MIC	6.25	50	>200
	MBC	12.5	100	>200
Staphylococcus aureus (37) (penicillin G susceptible)	MIC	1.56	1.56	1.56
	MBC	1.56	3.12	12.5
Staphylococcus aureus (38) (penicillin G resistant)	MIC	200	>200	>200
	MBC	>200	>200	>200
Streptococcus pyogenes (43)	MIB	0.05	0.05	0.10
	MBC	0.05	0.20	0.78
Streptococcus pneumoniae (15)	MIC	≥0.0125	0.0125–0.025	0.025
	MBC	0.0125	0.0125–0.025	0.025
Enterococci (27)	MIC	3.12–6.25	6.25	50
	MBC	>200	>200	>200

[a] MIC, Minimal inhibitory concentration; MBC, minimal bactericidal concentration.

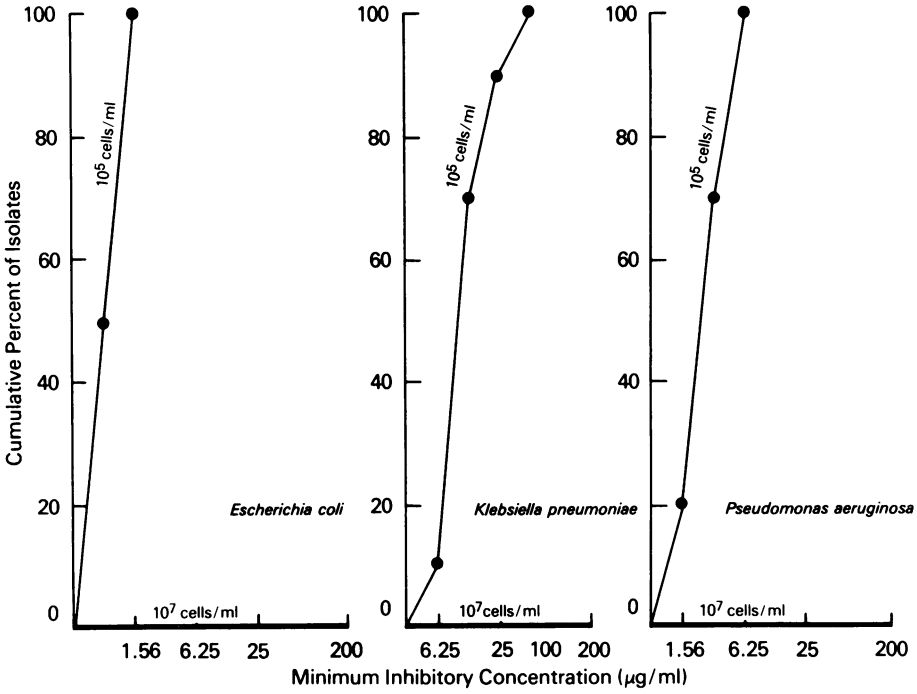

FIG. 2. *Effect of inoculum on activity of CI-867.*

pneumoniae. A concentration of 50 μg of CI-867 and 100 μg of mezlocillin or piperacillin per ml inhibited 75% of these isolates, whereas a concentration of 200 μg or greater of the other antibiotics per ml was required. CI-867, mezlocillin, and piperacillin were the most active antibiotics against isolates of *Enterobacter* spp. and *S. marcescens*. All antibiotics were quite active at concentrations expected to be achieved clinically against 90% of isolates of *Proteus mirabilis* and against 75% of isolates of *Proteus* spp. (indole positive), except ampicillin, which was inactive against the latter. Carbenicillin, ticarcillin, and piperacillin were the most active antibiotics against the latter, inhibiting 90% at concentrations of 12.5 and 25 μg/ml, respectively.

DISCUSSION

CI-867 is a new, semisynthetic penicillin with broad-spectrum activity in vitro. It is active against gram-positive cocci, except penicillin G-resistant *S. aureus*, and also against gram-negative bacilli. In general, its activity is similar to those of mezlocillin and piperacillin. It was especially active against *P. aeruginosa*. CI-867 was four times more active than ticarcillin and eight times more active than carbenicillin against 75% of these isolates. CI-867 was as active as piperacillin against isolates of *P. aeruginosa*, as active as mezlocillin against isolates of *K. pneumoniae*, and slightly less active than mezlocillin or piperacillin against isolates of *E. coli*. CI-867 has a broader spectrum than ampicillin, carbenicillin, or ticarcillin. Although CI-867 was active against a majority of isolates, some were resistant even at a concentration of 200 μg/ml, which is important clinically.

CI-867 was not active against strains which were multiply resistant (minimal inhibitory concentration, >200 μg/ml), although it is not known whether these are beta-lactamase-producing strains. Therefore, the method of resistance is uncertain. It should be noted that isolates from our patient population are generally more resistant to multiple antibiotics, possibly as a result of extensive use of both commerically available and investigational antimicrobial drugs

Inoculum size had a major effect on the activity of CI-867. It was inactive against all isolates of *E. coli*, *K. pneumoniae*, and *P. aeruginosa* when an inoculum of 10^7 cells per ml was used. This effect of inoculum variation has been reported in studies of other penicillins, including carbenicillin, azlocillin, and mezlocillin (4, 13, 14). One suggestion is that the larger inoculum contains a greater number of inherently resistant cells. Another suggestion is the loss of activity of CI-867 due to instability to beta-lactamase. Studies of beta-lactamase production in our

TABLE 2. *Comparative activity of antibiotics (in order of increasing activity) against gram-negative bacilli*

Organism (no. of isolates tested)	Drug	MIC (μg/ml) which inhibited the following percentage of isolates[a]:		
		50%	75%	90%
Pseudomonas aeruginosa (50)	CI-867	3.12	12.5	25
	Piperacillin	6.25	12.5	25
	Azlocillin	12.5	25	50
	Ticarcillin	25–50	50	100
	Mezlocillin	25–50	50	100
	Carbenicillin	50–100	100	>200
	Ampicillin	NT	NT	NT
Klebsiella pneumoniae (50)	CI-867	25	50	>200
	Mezlocillin	12.5	100	>200
	Piperacillin	25	100	>200
	Azlocillin	50	200	>200
	Ampicillin	50–100	200	>200
	Carbenicillin	>200	>200	>200
	Ticarcillin	>200	>200	>200
Escherichia coli (50)	Piperacillin	1.56	6.25	>200
	Mezlocillin	3.12	6.25	>200
	Ticarcillin	3.12	6.25	>200
	CI-867	3.12–6.25	12.5	>200
	Carbenicillin	3.12–6.25	12.5	>200
	Ampicillin	3.12–6.25	25	>200
	Azlocillin	6.25–12.5	12.5	>200
Serratia marcescens (50)	Piperacillin	3.12	6.25	50
	Mezlocillin	6.25	12.5	100
	Ticarcillin	6.25	12.5	>200
	CI-867	12.5	25	100
	Carbenicillin	12.5	25	>200
	Azlocillin	25	50	100
	Ampicillin	100	200	>200
Enterobacter spp. (50)	Mezlocillin	3.12–6.25	12.5	100
	Piperacillin	3.12	25	100
	CI-867	3.12	25	200
	Carbenicillin	6.25	50	>200
	Ticarcillin	6.25	50	>200
	Azlocillin	25	200	>200
	Ampicillin	>200	>200	>200
Proteus mirabilis (50)	Piperacillin	0.39	0.78	0.78
	Mezlocillin	0.78	1.56	1.56
	Carbenicillin	0.78	1.56	1.56
	Ticarcillin	0.78	1.56	1.56
	Ampicillin	1.56	1.56	3.12
	CI-867	3.12	3.12	6.25
	Azlocillin	3.12	6.25	6.25
Proteus spp. (21) (indole positive)	Carbenicillin	1.56	6.25	12.5
	Ticarcillin	3.12	12.5	25
	Piperacillin	1.56	3.12	100
	Mezlocillin	3.12	12.5	200
	CI-867	6.25	50	>200
	Azlocillin	25	100	>200
	Ampicillin	>200	>200	>200

[a] MIC, Minimal inhibitory concentration; NT, not tested.

group of isolates and studies of the stability of CI-867 to various beta-lactamases would be necessary to confirm this possibility but were not within the scope of this study.

The activity of CI-867 was not affected by the type of medium or the pH in tests on Mueller-Hinton broth. The first of these variables affects the activity of BLP-1654 and pirbenicillin (5, 6), and the second affects the activity of azlocillin and mezlocillin (4, 14).

CI-867 is active against those organisms that most frequently infect the compromised host, *P. aeruginosa*, *K. pneumoniae*, and *E. coli*, but is not active against penicillin G-resistant *S. aureus* (9).

In other studies, Cardenas et al. found that CI-867 has a spectrum of antibacterial activity greater than that of ticarcillin and comparable to that of piperacillin (7). In acute mouse protection tests, Heifetz and Sesnie found CI-867 to be markedly more effective than ticarcillin against *P. aeruginosa* and *K. pneumoniae* and more than twice as effective as piperacillin against the former (10).

The spectrum of activity and potency of CI-867 are such that it is not likely to be used as a single drug for treatment of presumptive infection. It might be useful in combination with a cephalosporin or an aminoglycoside. Special in vivo and pharmacological studies are needed to evaluate this possible use.

ACKNOWLEDGMENT

This work was supported in part by Public Health Service grant CA 05831 from the National Cancer Institute.

LITERATURE CITED

1. **Acred, P., D. M. Brown, E. T. Knudsen, G. N. Rolinson, and R. Sutherland.** 1967. New semi-synthetic penicillin active against *Pseudomonas pyocyanea*. Nature (London) **215**:25–30.
2. **Bodey, G. P.** 1972. Antibiotic therapy of infections in patients undergoing cancer chemotherapy. Antibiot. Chemother. (Basel) **18**:49–88.
3. **Bodey, G. P., and B. LeBlanc.** 1978. Piperacillin: in vitro evaluation. Antimicrob. Agents Chemother. **14**:78–87.
4. **Bodey, G. P., and T. Pan.** 1977. Mezlocillin: in vitro studies of a new broad-spectrum penicillin. Antimicrob. Agents Chemother. **11**:74–79.
5. **Bodey, G. P., V. Rodriguez, and S. Weaver.** 1976. Pirbenicillin, a new semi-synthetic penicillin with broad-spectrum activity. Antimicrob. Agents Chemother. **9**:668–674.
6. **Bodey, G. P., and D. Stewart.** 1971. In vitro studies of semisynthetic α-(substituted-ureido) penicillins. Appl. Microbiol. **21**:710–717.
7. **Cardenas, J. V., D. Pohlod, L. D. Saravolatz, R. Del Busto, K. Burch, T. Madhavan, E. J. Fischer, and E. L. Quinn.** 1979. Comparative in vitro activity of CI-867, a new semisynthetic penicillin, with ticarcillin and piperacillin, p. 363–365. *In* J. D. Nelson and C. Grassi (ed.), Current chemotherapy and infectious disease, vol. 1. American Society for Microbiology, Washington, D.C.
8. **Eickhoff, T. C.** 1973. Infectious complications in renal transplant patients. Transplant. Proc. **5**:1233–1238.
9. **Feld, R., G. P. Bodey, V. Rodriguez, and M. Luna.** 1974. Causes of death in patients with malignant lymphoma. Am. J. Med. Sci. **268**:97–106.
10. **Heifetz, C. L., and J. A. Sesnie.** 1979. CI-867, a novel penicillin derivative active against gram-negative pathogens, p. 367–370. *In* J. D. Nelson and C. Grassi (ed.), Current chemotherapy and infectious disease, vol. 1. American Society for Microbiology, Washington, D.C.
11. **Hinkle, A., B. M. LeBlanc, and G. P. Bodey.** 1980. In vitro evaluation of cefoperazone. Antimicrob. Agents Chemother. **17**:423–427.
12. **Inagaki, J., V. Rodriguez, and G. P. Bodey.** 1974. Causes of death in cancer patients. Cancer **33**:568–573.
13. **Standiford, H. C., A. C. Kind, and W. M. M. Kirby.** 1969. Laboratory and clinical studies of carbenicillin against gram-negative bacilli, p. 286–291. Antimicrob. Agents Chemother. 1968.
14. **Stewart, D., and G. P. Bodey.** 1977. Azlocillin: in vitro studies of a new semisynthetic penicillin. Antimicrob. Agents Chemother. **11**:865–870.