A NEW PENICILLIN WITH ANTI-KLEBSIELLA ACTIVITY: 3-(5-TETRAZOLYL) PENAM

GERALD P. BODEY, SUSANNE WEAVER and THERESA PAN

The Department of Developmental Therapeutics, The University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, 6723 Bertner, Houston, Texas 77030, U.S.A.

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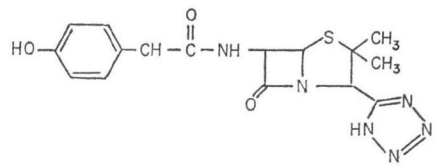
The *in vitro* activity of a new semi-synthetic penicillin, CP-35,587, 3-(5-tetrazolyl) penam, was investigated against 496 clinical isolates of gram-negative bacilli and 113 clinical isolates of gram-positive cocci. All of the gram-positive cocci were sensitive to CP-35,587 except penicillin G resistant isolates of *Staphylococcus aureus*. This antibiotic inhibited a majority of isolates of *Escherichia coli, Klebsiella* spp. and *Proteus mirabilis* at a concentration of 6.25 μg/ml. Also, approximately half of the isolates of *Serratia marcescens* and *Enterobacter* spp. were inhibited at a concentration of 12.5 μg/ml. CP-35,587 was inactive when high concentrations of organisms were used as inocula. CP-35,587 was more active than mezlocillin, azlocillin, amoxicillin, ticarcillin and carbenicillin against isolates of *K. pneumoniae*, but other penicillins were more active than CP-35,587 against other species of gram-negative bacilli.

The capability of producing semisynthetic penicillins has resulted in major advances in antibiotic therapy. The discovery of ampicillin and carbenicillin broadened the spectrum of penicillin activity to include gram-negative bacilli. These discoveries gave impetus to the synthesis of other penicillins with broad-spectrum activity. CP-35,587, 3-(5-tetrazolyl) penam is a new semi-synthetic penicillin in which the C3 carboxyl group of penicillin has been replaced with a 5-tetrazolyl group (Fig. 1). This penicillin derivative has been found to have broad spectrum activity in previous studies[1]. This report confirms these observations and indicates that it is especially active against *Klebsiella* spp.

Fig. 1. Chemical structure of CP-35,587, 3-(5-tetrazolyl) penam



Materials and Methods

Susceptibility tests were conducted on 496 clinical isolates of gram-negative bacilli and 113 clinical isolates of gram-positive cocci, using a dilution technique with an automatic microtiter system (Canalco: autotiter instruction manual). All gram-negative bacilli and *Staphylococcus aureus* isolates to be tested were incubated in MUELLER-HINTON Broth (pH 7.4) for 18 hours at 37°C. *Streptococcus pyogenes* and *S. pneumoniae* were incubated in Tryptose Phosphate Broth. Approximately 10^5 colony forming units (CFU/ml) were used as inoculum for gram-negative bacilli and *St. aureus*. For the remaining gram-positive cocci an inoculum of 10^6 CFU/ml was used for the *in vitro* sensitivety testing.

All gram-negative bacilli used in this study were cultured from blood specimens of patients who were hospitalized at this institution and had underlying malignant diseases. A total of 100 isolates

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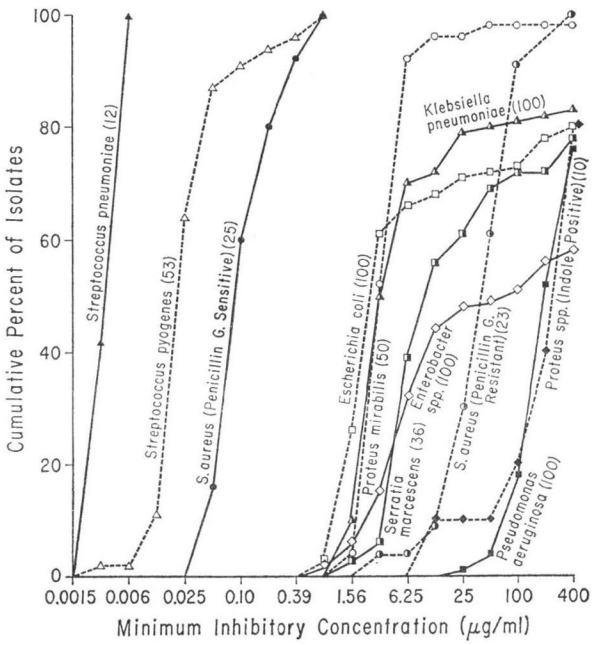
each of *Pseudomonas aeruginosa, Klebsiella* spp., *Enterobacter* spp. and *Escherichia coli*, 60 isolates of *Proteus* spp., and 36 isolates of *Serratia* spp. was used. All gram-positive cocci used in this study were cultured from specimens obtained from hospitalized patients, most of whom did not have cancer. A total of 53 isolates of *S. pyogenes*, 12 isolates of *S. pneumoniae* and 48 isolates of *St. aureus* was used. Isolates of *St. aureus* were divided according to their susceptibility to penicillin G. Those isolates which were inhibited by less than 0.10 μg/ml were selected as penicillin G sensitive and those isolates resistant to more than 25 μg/ml were selected as penicillin G resistant.

Organisms used for studies of the effect of inoculum size on the activity of CP-35,587 were incubated in MUELLER-HINTON Broth for 18 hours at 37°C. It was assumed that approximately 10^8 CFU/ml were present after incubation, which was subsequently confirmed by subculturing 0.1-ml aliquots on sheep blood agar and performing colony counts after 24 hours incubation at 37°C. Serial 10-fold dilutions of the broth culture were made, using MUELLER-HINTON Broth, so that 10^7 and 10^5 CFU/ml were used as inocula. An inoculum of 10^5 CFU/ml was used in all other studies of gram-negative bacilli. Studies of the effect of pH on the activity of CP-35,587 were conducted in MUELLER-HINTON Broth and the pH was adjusted to 6.4, 7.2 and 8.2 with phosphate buffer. Studies comparing the activity of CP-35,587 with mezlocillin, azlocillin, carbenicillin, ticarcillin and amoxicillin were conducted in MUELLER-HINTON Broth. Fifty isolates each of *E. coli, Klebsiella* spp., *P. aeruginosa, Enterobacter* spp. and *P. mirabilis*, 10 isolates of indole-positive *Proteus* spp. and 36 isolates of *S. marcescens* were used.

CP-35,587 was supplied by Pfizer Medical Research Laboratories, Groton, Connecticut. Azlocillin and mezlocillin (Bay 6905 and 1353) were supplied as powders by Delbay Pharmaceuticals Inc., Bloomfield, New Jersey. Carbenicillin, ticarcillin and amoxicillin were supplied by Beecham Pharmaceuticals, Bristol, Tennessee. All antibiotics were diluted serially in MUELLER-HINTON Broth or Tryptose Phosphate Broth. The minimum inhibitory concentration (MIC) was determined as no visible growth after 18 hours of incubation at 37°C. The minimum bactericidal concentration (MBC) was defined as the lowest concentration of drug which yielded less than 5 colonies on subculture to sheep blood agar (99% kill). A 0.01-ml-calibrated pipette was utilized to transfer the inoculum. All studies were performed simultaneously in triplicate.

Fig. 2. *In vitro* activity of CP-35,587 against gram-positive cocci and gram-negative bacilli

The numbers in parentheses indicate the number of isolates tested.



Results

Fig. 2 illustrates the activity of CP-35,587 *in vitro* against gram-positive cocci and gram-negative bacilli. Isolates of *S. pneumoniae*, *S. pyogenes* and penicillin G sensitive *St. aureus* were quite sensitive to CP-35,587. However, all of these isolates of *St. aureus* were inhibited by 0.1 μg/ml of penicillin G, whereas the MIC of CP-35,587 was 0.2~0.78 μg/ml for 40% of these isolates. Isolates of *St. aureus* resistant to penicillin G were also resistant to CP-35,587. This antibiotic was quite active against *E. coli*, *Klebsiella* spp. and *P. mirabilis*, in-

hibiting 66%, 70% and 92% of isolates at 6.25 μg/ml. At a concentration of 12.5 μg/ml, 56% of isolates of *S. marcescens* and 44% of isolates of *Enterobacter* spp. were inhibited. Indole-positive *Proteus* spp. and *P. aeruginosa* were resistant to CP-35,587. The MIC was also the MBC for most organisms.

The effect of inoculum size on the MIC and MBC were determined for 10 isolates each of *K. pneumoniae, E. coli* and *P. aeruginosa* (Fig. 3). When an inoculum of 10^7 CFU/ml was used, CP-35,587 failed to inhibit the growth of any of these organisms, even at concentration of 400 μg/ml. However, most of the isolates of *K. pneumoniae* and *E. coli* were quite sensitive to CP-35,587 when an inoculum of 10^5 CFU/ml was used.

Different media did not substantially affect the activity of this penicillin against most isolates of *K. pneumoniae* and *E. coli* (Fig. 4). However, some isolates of *P. aeruginosa* were more susceptible to CP-35,587 when nutrient broth was used and an few isolates were less susceptible when MUELLER-HINTON Broth was used. Changing the pH of MUELLER-HINTON broth from 6.4 to 8.0 did not affect the activity of this antibiotic against *K. pneumoniae* and *E. coli*. However, it was most active against isolates of *P. aeruginosa* at pH of 6.4.

The activity of CP-35,587 was compared to that of mezlocillin, azlocillin, amoxicillin, ticarcillin and carbenicillin against 50 isolates each of *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *Enterobacter* spp. and *P. mirabilis*, and 36 isolates of *S. marcescens* (Figs. 5~10). CP-35,587 was the most active penicillin against *K. pneumoniae*. At a concentration of 6.25 μg/ml, 76% of isolates were inhibited by CP-35,587 whereas 48% were inhibited by mezlocillin, the next most active penicillin. Mezlocillin was the most active penicillin against *E. coli*, *Enterobacter* spp. and *S. marcescens*. In general, CP-35,587 was more active than carbenicillin, ticarcillin and amoxi-

Fig. 3. Effect of inoculum size on activity of CP-35,587

Ten isolates each of *K. pneumoniae*, *E. coli* and *P. aeruginosa* were tested.

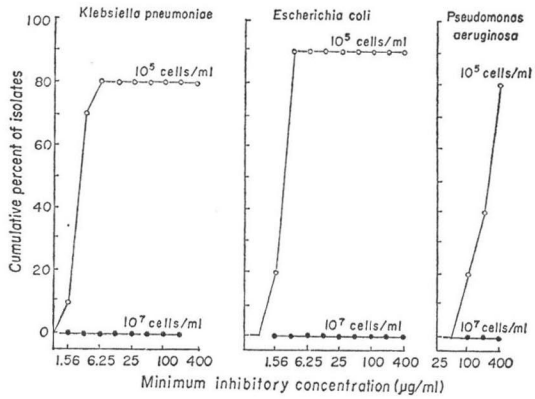


Fig. 4. Effect of media on activity of CP-35,587

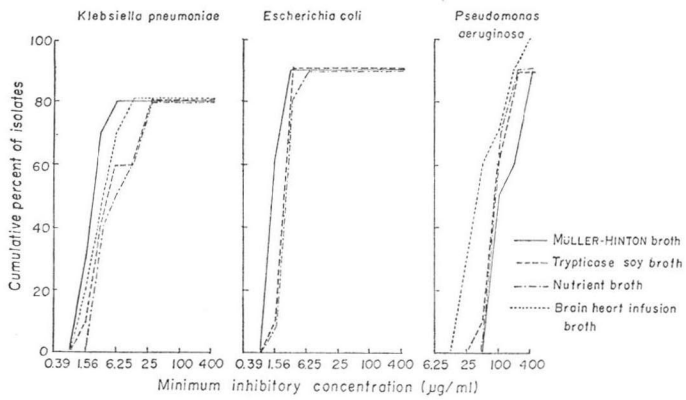


Fig. 5. Comparative activity of penicillins against 50 isolates of *Klebsiella* spp.

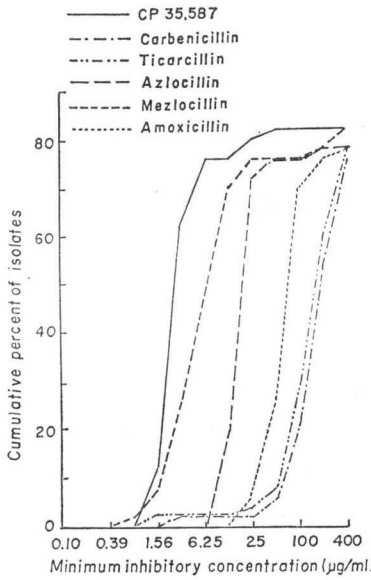


Fig. 6. Comparative activity of penicillins against 50 isolates of *E. coli*

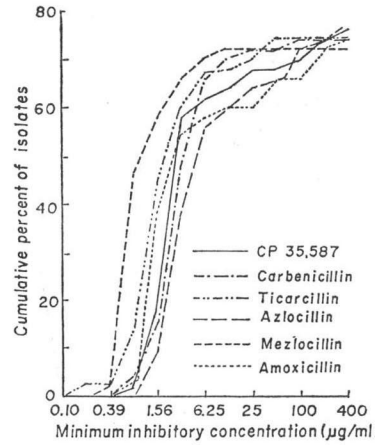


Fig. 7. Comparative activity of penicillins against 50 isolates of *Enterobacter* spp.

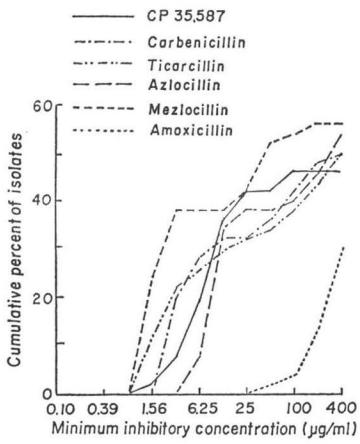
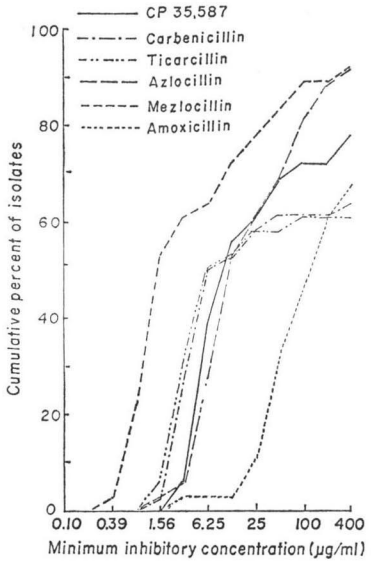


Fig. 8. Comparative activity of penicillins against 36 isolates of *S. marcescens*



cillin against these organisms. This penicillin was the least active against *P. mirabilis* and was only slightly more active than amoxicillin against 10 isolates of indole-positive *Proteus* spp. (not illustrated). All of the other penicillins had greater activity than CP-35,587 against indole-positive *Proteus* spp. Azlocillin was the most active penicillin against isolates of *P. aeruginosa*. CP-35,587 was less active than carbenicillin, and only 32% of isolates were inhibited by 100 μg/ml.

Discussion

CP-35,587 is a new semi-synthetic penicillin with broad-spectrum activity *in vitro*. It is active against gram-positive cocci, except penicillin G resistant *St. aureus* and also against many gram-

Fig. 9. Comparative activity of penicillins against 50 isolates of *P. aeruginosa*

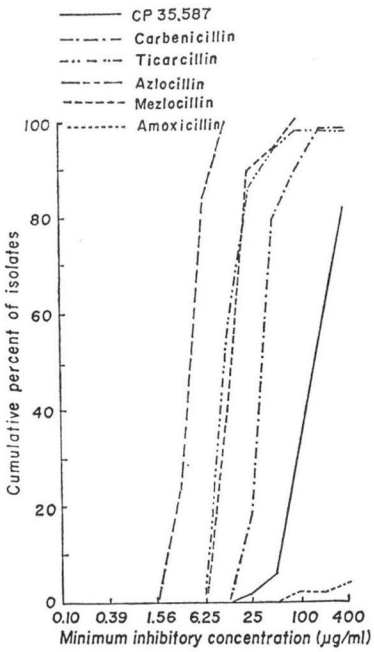
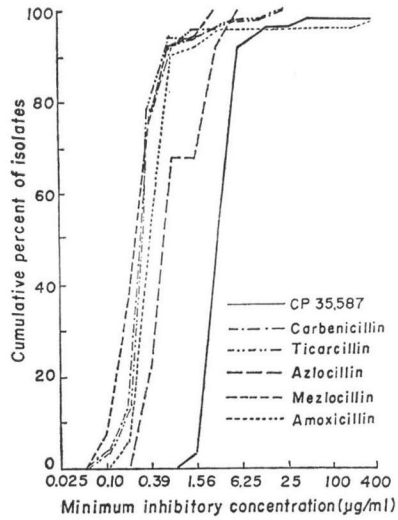


Fig. 10. Comparative activity of penicillins against 50 isolates of *P. mirabilis*



negative bacilli. A major advantage of this antibiotic is its superior activity against isolates of *Klebsiella* spp. A concentration of 6.25 μg/ml inhibited 70% of isolates. CP-35,587 was more active against *Klebsiella* spp. than any of the other penicillins tested. Previous studies also indicated that it was as active as cephalexin against these organisms[1]. Although CP-35,587 had broad-spectrum activity against most other gram-negative bacilli, other penicillins had greater activity. For example, mezlocillin was more active against most Enterobacteriaceae and azlocillin was more active against *P. aeruginosa*.

The activity of CP-35,587 against gram-negative bacilli was affected by the size of the inoculum. A concentration of 400 μg/ml failed to inhibit a large inoculum of organisms which were inhibited by lower concentrations when a smaller inoculum was used. This effect of inoculum size has been observed with other penicillins, including ticarcillin, carbenicillin and mezlocillin[2~4]. It has been proposed that all cells in an inoculum are not uniformly susceptible to these penicillins and that a large inoculum includes more cells which are inherently resistant to the antibiotic. The clinical significance of this observation is not clear.

The frequency of serious *Klebsiella* infections in hospitalized patients is increasing in recent years. This is especially true among patients with compromised host defense mechanisms. Many of these infections are caused by strains which are resistant to currently available antibiotics or respond poorly to therapy[5]. Therefore, new antibiotics of potential value should be investigated. CP-35,587 is a penicillin with substantial activity against *Klebsiella* spp. and should be examined further for its potential clinical application.

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