

In Vitro Studies of α -Carboxyl-3-Thienylmethyl Penicillin, a New Semisynthetic Penicillin

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The activity of a new semisynthetic penicillin, α -carboxyl-3-thienylmethyl penicillin (BRL-2288) was determined against 535 clinical isolates of gram-negative bacilli, by using the tube dilution technique. Nearly 80% of isolates of *Proteus* spp. were inhibited by 3.12 μ g or less of this antibiotic per ml. BRL-2288 was as active as ampicillin against *Escherichia coli*. It was slightly more active than carbenicillin or 6-(D- α -sulfoaminophenylacetamido)-penicillanic acid against *Pseudomonas* sp., with over half of the isolates being inhibited by 50 μ g or less of BRL-2288 per ml. Isolates of *Klebsiella* sp. were routinely resistant to this antibiotic. The drug was bactericidal against most sensitive organisms. BRL-2288 was less active against large inocula. A strain of *Pseudomonas* sp. which developed resistance to carbenicillin also developed resistance to BRL-2288 simultaneously.

Gram-negative bacilli are responsible for an increasing number of infections occurring in hospitalized patients (2, 10, 12). Although most of these infections are caused by *Escherichia coli* and *Klebsiella* sp., a substantial number are caused by *Pseudomonas* sp. and *Proteus* sp. *Pseudomonas* sp. infections are very common in patients with leukemia, metastatic cancer, cystic fibrosis, and extensive burns (14, 19).

For many years the polymyxins were the only antibiotics available for the treatment of *Pseudomonas* sp. infections. Although these antibiotics are very active against *Pseudomonas* sp. in vitro, they are only minimally effective against systemic infections in patients with impaired host defense mechanisms. The aminoglycoside antibiotic, gentamicin sulfate, is active in vitro against most gram-negative bacilli, including *Pseudomonas* sp. Although this drug has been effective in the treatment of many *Pseudomonas* sp. infections, it has been of limited value in patients with granulocytopenia (6, 20).

Recently, several penicillin derivatives have been synthesized which are active against gram-negative bacilli, including *Pseudomonas* sp. (1, 7, 18). Carbenicillin is the first semisynthetic penicillin with antipseudomonal activity to undergo clinical investigation. This antibiotic is effective even in patients with impaired host defenses and severe granulocytopenia (5, 8, 21). It is also active against indole-negative and indole-positive *Proteus* spp. and some *E. coli* and *Enterobacter* spp. However, because it is only marginally active

against *Pseudomonas* sp., very large doses are required for the treatment of systemic infections. Another penicillin, 6-(D- α -sulfoaminophenylacetamido)-penicillanic acid (BLP-1462) has undergone preliminary investigation and appears to be comparable to carbenicillin (4).

A new semisynthetic penicillin, α -carboxyl-3-thienylmethyl penicillin (BRL-2288) has recently been made available for laboratory investigation (Fig. 1). The drug is a disodium salt which is soluble in water. Refrigerated aqueous solutions are stable for about 1 week. The in vitro studies presented in this report indicate that BRL-2288 is active against *Proteus* spp., *E. coli*, and *Pseudomonas* sp.

MATERIALS AND METHODS

Sensitivity tests were conducted on 535 clinical isolates of gram-negative bacilli by using the tube dilution technique (13). The organisms were inoculated into Mueller-Hinton broth (BBL) and incubated at 37 C for 18 hr. A 0.1-ml sample of a 10^{-4} dilution of this broth (approximately 10^8 colony-forming units) was used as the inoculum for sensitivity testing. The α -carboxyl-3-thienylmethyl penicillin used in these studies was supplied as BRL-2288 by Beecham Pharmaceuticals, Division of Beecham, Inc., Clifton, N.J. It was dissolved in Mueller-Hinton broth to a concentration of 400 μ g/ml. Twofold serial dilutions of BRL-2288 were made with Mueller-Hinton broth, and the minimum inhibitory concentration (MIC) was determined after incubation at 37 C for 18 hr. All tubes containing trace growth or no discernable growth were subcultured on sheep blood-agar. The drug was con-

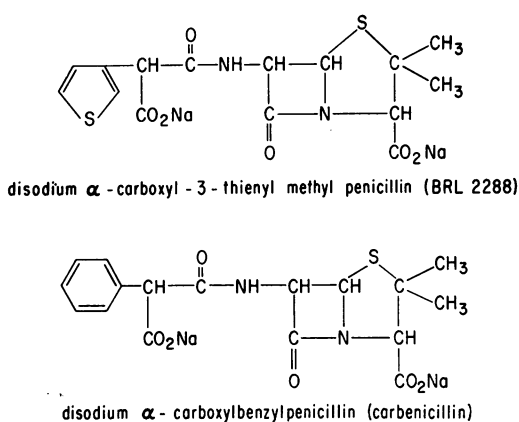


FIG. 1. Chemical structure of α -carboxyl-3-thienyl-methyl penicillin.

sidered to be bactericidal against those isolates which yielded less than 10 colonies on subculture of the tube containing the MIC.

All organisms used in this study were cultured from blood specimens obtained from patients between December 1966 and March 1970. The majority of these patients were hospitalized at this institution and had an underlying malignant disease. A total of 90 isolates of *Proteus* spp., 100 isolates of *E. coli*, 45 isolates of *Enterobacter* spp., 50 isolates of *Serratia* sp., 100 isolates of *Klebsiella* sp., and 150 isolates of *Pseudomonas* sp. were studied.

RESULTS

The activity of BRL-2288 against gram-negative bacilli is shown in Fig. 2. The drug was most active against *Proteus* spp., nearly 80% of these isolates having an MIC of 3.12 $\mu\text{g/ml}$ or less. BRL 2288 inhibited 83% of isolates of *P. mirabilis* and 70% of isolates of indole-positive *Proteus* spp. at this concentration. Over half of the isolates of *E. coli* had an MIC of 6.25 $\mu\text{g/ml}$ or less, and the majority of isolates of *Pseudomonas* sp. were inhibited by 50 $\mu\text{g/ml}$ or less of the antibiotic. The majority of isolates of *Serratia* sp. and *Klebsiella* sp. were resistant to BRL-2288.

BRL-2288 was bactericidal against 74% of the sensitive isolates of *E. coli*, 74% of *Enterobacter* spp., 64% of *Proteus* spp., and 63% of *Pseudomonas* sp. It was bactericidal against 42% of the few sensitive isolates of *Klebsiella* sp. and 25% of *Serratia* sp.

The effect of inoculum size on the MIC of BRL-2288 was determined against 10 isolates each of *Pseudomonas* sp., *E. coli*, and *Serratia* sp., and 5 isolates of *Proteus* sp. (Fig. 3). The inocula used were 10^{-3} , 10^{-4} , 10^{-5} , and 10^{-6} dilutions of an 18-hr broth culture of the test organisms (containing approximately 10^8 colony-

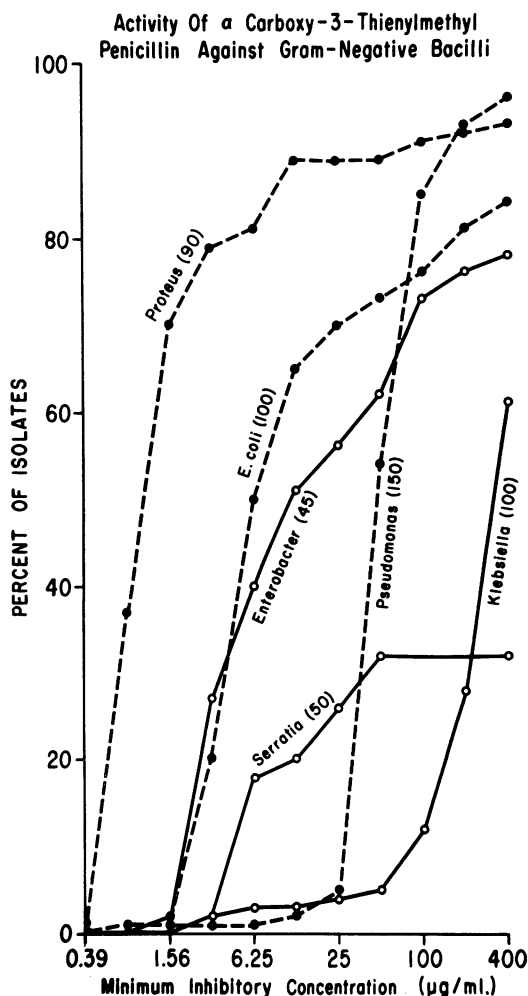


FIG. 2. *In vitro* activity of α -carboxyl-3-thienyl-methyl penicillin against gram-negative bacilli, with the tube-dilution technique. The MIC is plotted on a \log_2 scale and the cumulative percentage of sensitive strains is shown. The numbers in parentheses indicate the number of isolates tested.

forming units/ml). In general, the MIC was higher when the largest inoculum was used. This was especially true for *Pseudomonas* sp. and *Serratia* sp. There was no substantial difference between the 10^{-4} and 10^{-5} dilutions.

The activity of BRL-2288 was compared simultaneously with carbenicillin and 6-(D- α -sulfoaminophenylacetamido)-penicillanic acid, by using 50 isolates each of *E. coli*, *Proteus* spp., and *Pseudomonas* sp. Ampicillin was also used against *E. coli* and *Proteus* spp. BRL-2288 was as active as ampicillin against *E. coli* and substantially more active than 6-(D- α -sulfoaminophenylacetamido)-

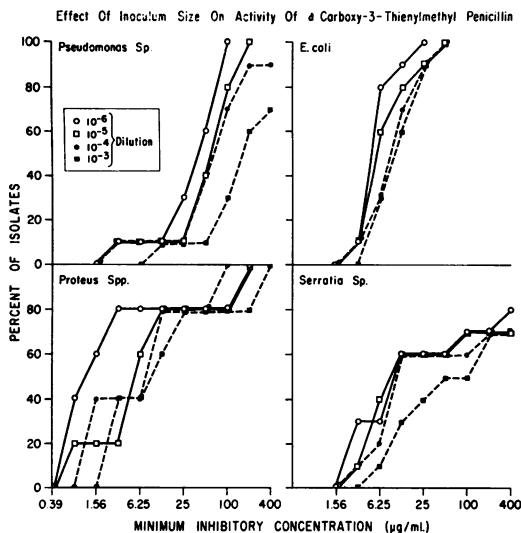


FIG. 3. Effect of inoculum size on the activity of α -carboxyl-3-thienylmethyl penicillin against *Pseudomonas* sp., *E. coli*, *Proteus* spp. and *Serratia* sp.

penicillanic acid (Fig. 4). Three isolates were substantially more sensitive (a fourfold or greater difference in the MIC) to BRL-2288 than to ampicillin, and six isolates were substantially more sensitive to ampicillin.

BRL-2288 was the most active drug against *Pseudomonas* sp. (Fig. 5). At a concentration of 50 $\mu\text{g/ml}$ or less, BRL-2288 was effective against 80% of the isolates, whereas the other two semisynthetic penicillins were effective against less than 30%. Thirteen isolates were substantially more sensitive to BRL-2288 than to 6-(D- α -sulfoaminophenylacetamido)-penicillanic acid, and seven isolates were substantially more sensitive to BRL-2288 than to carbenicillin. None of the isolates was substantially more sensitive to the other two penicillins than to BRL-2288.

BRL-2288 was the most active antibiotic against both indole-negative and indole-positive *Proteus* spp. (Fig. 6). At a concentration of 0.78 $\mu\text{g/ml}$ or less, BRL-2288 was active against nearly 60% of the isolates of *P. mirabilis*, whereas the other three penicillins were active against less than 30%. The difference in activity was more striking against indole-positive *Proteus* spp. At a concentration of 1.56 $\mu\text{g/ml}$ or less, BRL-2288 and carbenicillin were active against over 80% of the isolates, whereas the other two penicillins were active against less than 25%. Three isolates of *Proteus* spp. were substantially more sensitive to BRL-2288 than to carbenicillin, and two isolates were substantially more sensitive to carbenicillin.

The activity of BRL-2288 and 6-(D- α -sulfo-

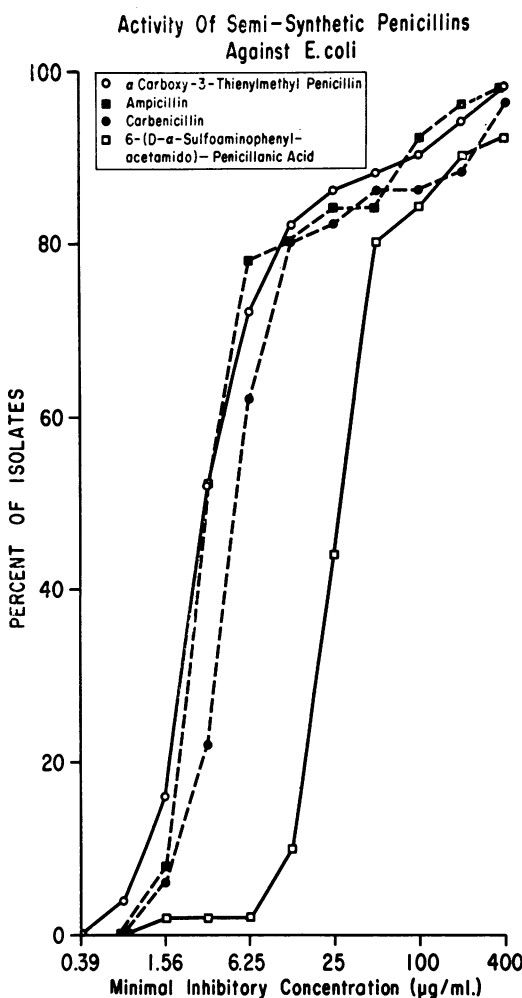


FIG. 4. Activity of semisynthetic penicillins against 50 clinical isolates of *E. coli*.

aminophenylacetamido)-penicillanic acid was determined against a strain of *Pseudomonas* sp. which had developed resistance to carbenicillin (Fig. 7). *Pseudomonas* sp. was cultured from the blood of a patient who was treated with 30 g of carbenicillin for 12 days. The patient failed to respond, and *Pseudomonas* sp. of the same pyocine type was cultured from his blood on several occasions between days 7 and 12. This strain of *Pseudomonas* sp. developed resistance in vitro to all three semisynthetic penicillins.

DISCUSSION

BRL-2288 is a new semisynthetic penicillin which is active in vitro against most clinical isolates of *E. coli*, *Proteus* spp., and *Pseudomonas*

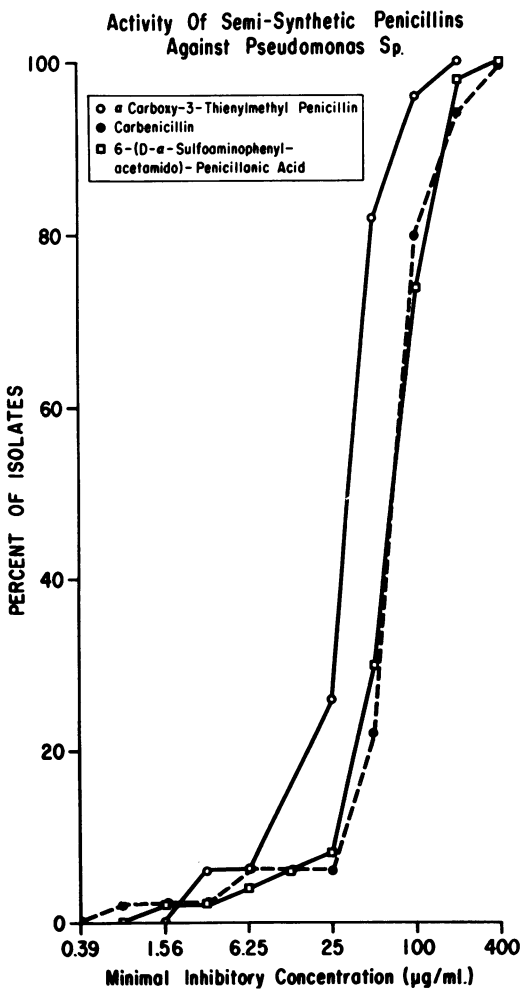


FIG. 5. Activity of semisynthetic penicillins against 50 clinical isolates of *Pseudomonas* sp.

sp. It is active against some isolates of *Enterobacter* spp. and a few isolates of *Serratia* sp., but it is ineffective against *Klebsiella* sp. The MIC varies with the size of the inoculum, which is also true for carbenicillin and 6-(D- α -sulfoaminophenylacetamido)-penicillanic acid (1, 7).

BRL-2288 compares favorably with other semisynthetic penicillins which are active against gram-negative bacilli. It is as active as ampicillin against *E. coli*, and, with few exceptions, organisms resistant to one of these antibiotics are resistant to the other. BRL-2288 is more active than carbenicillin or 6-(D- α -sulfoaminophenylacetamido)-penicillanic acid against *E. coli*. BRL-2288 is the most active of these semisynthetic penicillins against *Pseudomonas* sp. and *Proteus* spp. Like carbenicillin, it is equally active against indole-negative and indole-positive *Proteus* spp.

Most isolates of *Klebsiella* sp. have been resistant to all of the semisynthetic penicillins, and superinfections with this organism have been a problem in patients receiving carbenicillin (5, 15). However, BRL-2288 and carbenicillin are active against some isolates of *Enterobacter* spp. This activity is the opposite of the cephalosporin antibiotics which are effective against *Klebsiella* sp. but inactive against *Enterobacter* spp. (11). In general, the semisynthetic penicillins have had little or no activity against *Serratia* sp., and superinfections with this organism have been a problem at our institution (5). Clinical isolates of nonpigmented *Serratia* sp. obtained from patients at our institution are highly resistant to antibiotics, being routinely sensitive only to gentamicin sulfate (6). However, at some institutions, as high as 80% of the isolates of *Serratia* sp. have been sensitive to carbenicillin (17).

A substantial number of carbenicillin-resistant isolates of *Pseudomonas* sp. have been found at

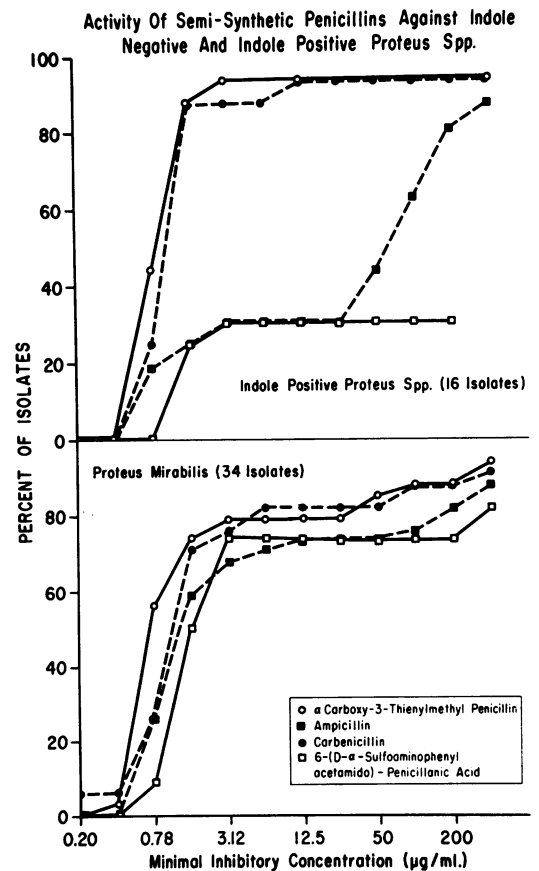


FIG. 6. Activity of semisynthetic penicillins against 34 clinical isolates of *P. mirabilis* and 16 clinical isolates of indole-positive *Proteus* spp.

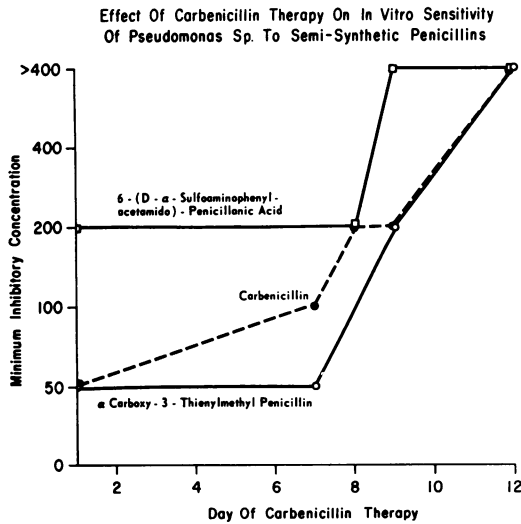


FIG. 7. Susceptibility of four successive isolates of *Pseudomonas* sp. cultured from blood specimens of a patient during treatment with carbenicillin.

some institutions where this drug has been used (3, 9). The emergence of resistant strains and clinical failures have been associated with the use of suboptimal doses of this drug (15). At our institution, only one patient with *Pseudomonas* sp. infection has failed to respond because the organism became resistant to carbenicillin. This organism also became resistant in vitro to both BRL-2288 and 6-(D- α -sulfoaminophenylacetamido)-penicillanic acid, suggesting that these new penicillins will not be effective against carbenicillin-resistant *Pseudomonas* sp. It has been suggested that resistance to carbenicillin is due to the production of a specific carbenicillinase (16). This is unlikely in our case, since the organism simultaneously developed resistance to the other two penicillins which have different chemical structures.

Carbenicillin has been effective in the treatment of infections caused by *Pseudomonas* sp. and *Proteus* spp., The only major disadvantage is the large dosage required for effective therapy. The high sodium load sometimes causes congestive heart failure in patients with marginal cardiac and renal function (15). These in vitro studies suggest that BRL-2288 may be useful in the treatment of infections caused by *Pseudomonas* sp., *Proteus* spp., and some *E. coli*. Since BRL-2288 is slightly more active than carbenicillin in vitro, it may be effective clinically at lower doses. Hopefully, this drug will eliminate some of the problems associated with the administration of carbenicillin.

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