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Comparative In Vitro Study of Temocillin (BRL 17421), a New Penicillin

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The activity of temocillin (BRL 17421), a new penicillin, was tested in vitro against 653 isolates of gram-negative bacilli and gram-positive cocci. The drug was compared with other β-lactam antibiotics and tobramycin. It inhibited the majority of gram-negative bacilli tested except for *Pseudomonas aeruginosa* and *Acinetobacter calcoaceticus*, which were highly resistant. It was active against more than 50% of the multiresistant strains tested. Temocillin was more active than mezlocillin against most gram-negative bacilli and more active than moxalactam, ceftriaxone, and ceftazidime against *Enterobacter* spp. In general, it was slightly less active than the other drugs tested and had no activity against the gram-positive cocci. There was no significant change in drug activity when pH and medium were varied, and the effect of serum binding was minimal. There was no significant inoculum effect when the size of the inoculum was increased from 10^4 to 10^6 organisms per ml.

In the past decade, numerous structural modifications of the side chains of the penicillin, cephalosporin, and cephamycin nuclei have been introduced. Many of these new β-lactam antibiotics have a broader spectrum of activity against members of the *Enterobacteriaceae* family and anaerobes, including *Bacteroides fragilis* (2–4, 13). Several of these compounds, including cefoxitin, cefuroxime, and ceftazidime, are less susceptible to degradation by β-lactamases (10, 11). Other compounds, such as moxalactam, have been found not only to be resistant to hydrolysis by β-lactam but, in fact, to inhibit some of these enzymes (5).

Temocillin (BRL 17421), 6β-(2-carboxy-2-thien-3-yl acetamido)-6α-methoxypenicillanic disodium salt, is a novel semisynthetic β-lactam in which a methoxy group has been introduced at the 6α position in the nucleus (Fig. 1). This compound is active against a wide range of gram-negative bacteria and shows great stability to β-lactamases produced by these organisms (12). In this study, temocillin was tested for its in vitro efficacy against clinical isolates obtained from patients in a hospital where antibiotics are used extensively and, as a consequence, where many of these organisms are resistant to multiple antibiotics.

MATERIALS AND METHODS

A total of 492 clinical isolates of gram-negative bacilli and 109 isolates of gram-positive cocci were tested. All gram-negative bacilli, with the exception of a few indole-positive *Proteus* spp., were isolated from blood cultures obtained from cancer patients at this institution in the past 10 years. Isolates of gram-positive cocci and a few isolates of indole-positive *Proteus* spp. were obtained from cultures taken from various body sites of hospitalized patients, some of whom did not have cancer. All isolates were maintained in stock by lyophilization or ultrafreezing methods. Organisms were tested in duplicate simultaneously and included 100 isolates each of *Escherichia coli* and *Klebsiella* spp., 50 isolates of *Pseudomonas aeruginosa, Serratia marcescens,* and *Enterobacter* spp., 45 isolates of *Proteus mirabilis*, 29 isolates of indole-positive *Proteus* spp., 25 isolates of *Acinetobacter calcoaceticus*, and 18 isolates of *Citrobacter* spp. Gram-positive organisms consisted of 50 isolates of *Staphylococcus aureus*, 25 isolates of *Streptococcus pyogenes*, 9 isolates of *Streptococcus pneumoniae*, and 25 isolates of enterococci. The *S. aureus* isolates were selected on the basis of a minimum inhibitory concentration (MIC) of ≤0.01 μg/ml (penicillin G susceptible) or an MIC of ≥25 μg/ml (penicillin G resistant). Appropriate dilutions were made so that the final concentration of organisms was 10^5 cells per ml (10^6 cells per ml for *S. pyogenes* and *S. pneumoniae*). The concentration and purity of all isolates were confirmed by plate counting.

A group of multiresistant aerobic gram-negative bacilli was also tested against temocillin. The organisms studied were 14 isolates of *Klebsiella pneumoniae*, 13 isolates of *Enterobacter* spp., 8 isolates of *S. marcescens*, 7 isolates of *P. aeruginosa*, 4 isolates of *Achromobacter* spp., 3 isolates of *Aeromonas hydrophila*, 2 isolates of *E. coli*, and 1 isolate of *Citrobacter freundii*. All organisms selected for this part of the study were resistant to at least two different antibiotic classes as defined by the following MICs (micrograms

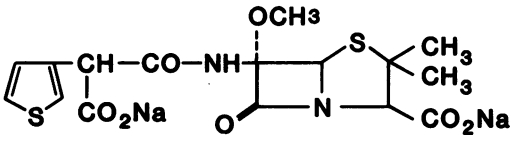

FIG. 1. Chemical structure of temocillin, disodium 6β-(2-carboxy-2-thien-3-yl acetamido)-6α-methoxypenicillanate.

per milliliter): for cephalothin, cefoxitin, and cefamandole, >100; for trimethoprim, >16; for sulfamethoxazole, 304; for tobramycin, >50; for carbenicillin, >400; and for chloramphenicol, >50.

Antibiotics were supplied as follows: temocillin by Beecham Laboratories, Bristol, Tenn.; moxalactam by Eli Lilly & Co., Indianapolis, Ind.; thienamycin by Merck & Co., Rahway, N.J.; ceftriaxone by Hoffmann-La Roche, Inc., Nutley, N.J.; ceftazidime by Glaxo Research Ltd., Ft. Lauderdale, Fla.; and mezlocillin by Delbay Pharmaceuticals, Inc., Florham Park, N.J.

Serial antibiotic concentrations were prepared manually each day of testing in Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) or tryptose phosphate broth (Difco) and dispensed automatically by a Dynatech MIC-2000 dispenser (Dynatech Laboratories, Inc. Alexandria, Va.) for susceptibility testing.

Automatic inoculations were performed by a Dynatech MIC-2000 inoculator in susceptibility tests to temocillin alone (except with isolates of *S. pyogenes* and *S. pneumoniae*), in comparative studies, in studies of the effect of human serum, and in studies of pH and medium variation. The inoculator pins delivered 0.0015 ml of a 1:10 dilution of broth cultures incubated at 37°C for 18 h into microtiter plate wells containing 0.10 ml of medium with various antibiotic concentrations to achieve a final inoculum concentration of approximately 1.5×10^5 cells per ml.

All isolates were tested in Mueller-Hinton broth, with the exception of isolates of *S. pyogenes* and *S. pneumoniae*, which were tested in tryptose phosphate broth to enhance growth. In studies of the effect of human serum, isolates were tested with a 1:1 mixture of commercially prepared pooled human serum (GIBCO Diagnostics, Madison, Wis.) and Mueller-Hinton broth as a diluent.

In performing susceptibility testing with isolates of *S. pyogenes* and *S. pneumoniae* and in studies of inoculum variation, we inoculated the microtiter plates manually. A 0.05-ml sample of a 1:100 dilution of broth cultures of *S. pyogenes* and *S. pneumoniae* incubated in a 5% CO_2 incubator at 37°C for 18 h was dripped by a calibrated pipette dropper into wells containing 0.05 ml of various antibiotic concentrations to achieve a final inoculum concentration of approximately 10^6 cells per ml. In studies of inoculum variation, we adjusted the inoculum concentration by preparing various dilutions (10^2, 10^4, 10^6) of broth cultures incubated at 37°C for 18 h and dripping 0.05 ml into wells containing 0.05 ml of medium containing various antibiotic concentrations.

The same 10 strains each of *K. pneumoniae*, *E. coli*, and *P. mirabilis* were used for these studies with temocillin. We determined the effect of pH on drug activity by adjusting the pH of Mueller-Hinton broth to 6.4, 7.2, and 8.0 with 1 N NaOH or 1 N HCl. The effect of medium on the activity of BRL temocillin 17421 was studied with Mueller-Hinton broth, Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.), brain heart infusion broth, and nutrient broth. We determined the effect of serum binding on the MICs of 30 isolates, 10 each of *P. mirabilis*, *E. coli*, and *K. pneumoniae*, in duplicate using human serum mixed as a diluent 1:1 with Mueller-Hinton broth. These same isolates were tested simultaneously in the same plate with Mueller-Hinton broth as a diluent.

The MIC was defined as the lowest concentration of drug that suppressed visible growth after incubation at 37°C for 18 h for gram-negative bacilli, isolates of *S. aureus*, and enterococci and after incubation at 37°C in a CO_2 incubator for 24 h for isolates of *S. pyogenes* and *S. pneumoniae*. The minimal bactericidal concentration (MBC) was defined as the lowest concentration of drug that yielded fewer than five colonies on subculture to sheep blood agar (99.5% kill) after incubation at 37°C for 18 h. A micropipetting device was used to deliver 10 μl for subculture. *Enterobacter cloacae* ATCC 13047, *Proteus vulgaris* ATCC 6380, and *K. pneumoniae* ATCC 27736 were included as controls during each procedure.

RESULTS

The in vitro activity of temocillin is summarized in Table 1. The compound was active against all of the aerobic gram-negative bacilli tested except for *P. aeruginosa*, *A. calcoaceticus*, and *S. marcescens*. A concentration of 12.5 μg/ml inhibited 97% of the *E. coli* isolates, 95% of the *K. pneumoniae* isolates, 90% of the *Enterobacter* spp. isolates, and 94% of the *Citrobacter* spp. isolates. Both *P. mirabilis* and indole-positive *Proteus* spp. were highly susceptible; 99% of the isolates were inhibited by a concentration of 1.56 to 3.12 μg/ml. Of the *S. marcescens* isolates, 70% were inhibited by a concentration of 25 μg/ml. The most resistant organisms against temocillin were *A. calcoaceticus* and *P. aeruginosa*. At concentrations of 200 μg/ml, 34 and 40%, respectively, of the isolates tested were inhibited. Temocillin was bactericidal at concentrations expected to be achieved clinically against a majority of the isolates of all organisms except *A. calcoaceticus*, *S. marcescens*, *Enterobacter* spp., and *P. aeruginosa*. A concentration of 12.5 μg/ml was inhibitory for ≥90% of the *Enterobacter* spp. isolates, but bactericidal activity for 70% of the isolates required concentrations of 100 μg/ml. Similarly high concentrations were needed to achieve bactericidal activity against *S. marcescens*.

The activity of temocillin against members of the *Enterobacteriaceae* family was compared with that of moxalactam, thienamycin, ceftriaxone, ceftazidime, mezlocillin, and tobramycin (Table 2). Temocillin was as effective as tobramycin against indole-positive *Proteus* spp. and

TABLE 1. In vitro activity of temocillin

Organism (no. tested)	Concn (μg/ml) required to inhibit the following % of isolates:					
	50		70		90	
	MIC	MBC	MIC	MBC	MIC	MBC
E. coli (100)	3.12	6.25	6.25	12.5	12.5	100
K. pneumoniae (100)	3.12	6.25	6.25	12.5	12.5	25
Enterobacter spp. (75)	6.25	50	12.5	100	12.5	>100
S. marcescens (50)	25	>50	25	>50	>50	>50
P. mirabilis (45)	1.56	1.56	1.56	1.56	1.56	3.12
Proteus spp., indole positive (29)	1.56	3.12	1.56	6.25	3.12	25
Citrobacter spp. (18)	3.12	12.5	6.25	12.5	12.5	50
A. calcoaceticus (25)	>200	>200	>200	>200	>200	>200
P. aeruginosa (50)	>200	>200	>200	>200	>200	>200
S. aureus (25), penicillin G susceptible	>50	>50	>50	>50	>50	>50
S. aureus (25) penicillin G resistant	>50	>50	>50	>50	>50	>50
S. pyogenes (25)	>50	>50	>50	>50	>50	>50
S. pneumoniae (9)	>50	>50	>50	>50	>50	>50
Enterococcus (25)	>50	>50	>50	>50	>50	>50

P. mirabilis. Against *Citrobacter* spp., it was more active than all other antibiotics tested except moxalactam and thienamycin. Temocillin was less active than thienamycin and tobramycin against *Enterobacter* spp. Compared with mezlocillin, temocillin was four times more active against *E. coli* and eight times more active against *K. pneumoniae*. However, it was less active than the other drugs tested against these two organisms. Temocillin exhibited a moderate degree of activity against *S. marcescens*; 76% of isolates were inhibited at a concentration of 25 μg/ml. Ceftriaxone was the most active drug against this organism, inhibiting 92% of isolates at 0.39 μg/ml. The next most active drugs were ceftazidime and moxalactam, which inhibited 94

TABLE 2. Comparative in vitro activity against gram-negative bacilli

Organism (no. tested)	% of isolates inhibited	Antibiotic concn (μg/ml)						
		Temocillin	Moxalactam	Thienamycin	Ceftriaxone	Ceftazidime	Mezlocillin	Tobramycin
E. coli (50)	50	3.12	0.10	0.10	<0.05	0.20	1.56	1.56
	75	6.25	0.20	0.20	<0.05	0.20	25	1.56
	90	6.25	0.20	0.20	<0.05	0.20	25	3.12
K. pneumoniae (50)	50	1.56	0.20	0.20	<0.05	0.20	25	0.39
	75	3.12	0.20	0.39	0.10	0.20	50	0.39
	90	6.25	0.39	0.39	0.10	0.39	50	0.78
P. aeruginosa (50)	50	>200	12.5	1.56	25	1.56	25	0.20
	75	>200	25	1.56	100	3.12	50	0.39
	90	>200	25	3.12	200	6.25	100	0.39
Enterobacter spp., (50)	50	6.25	3.12	0.39	3.12	6.25	25	0.78
	75	12.5	12.5	0.78	50	25	200	0.78
	90	12.5	25	1.56	>100	100	>200	6.25
S. marcescens (50)	50	25	0.39	1.56	0.20	0.39	6.25	3.12
	75	25	0.78	3.12	0.39	0.78	6.25	3.12
	90	>50	0.78	3.12	0.39	0.78	>100	6.25
P. mirabilis (45)	50	1.56	0.10	1.56	0.05	0.20	0.78	0.78
	75	1.56	0.10	3.12	0.05	0.05	0.78	1.56
	90	1.56	0.10	3.12	0.05	0.05	1.56	1.56
Proteus spp., indole positive (29)	50	1.56	0.10	3.12	0.78	0.20	6.25	0.39
	75	1.56	0.20	3.12	50	1.56	25	0.78
	90	3.12	0.20	3.12	>100	25	>100	3.12
A. calcoaceticus (25)	50	>200	50	0.39	12.5	6.25	25	0.78
	75	>200	50	0.39	25	12.5	50	1.56
	90	>200	100	1.56	25	12.5	>100	12.5
Citrobacter spp., (18)	50	3.12	0.20	0.39	0.39	0.39	25	1.56
	75	6.25	0.39	0.78	0.78	0.78	50	1.56
	90	12.5	6.25	0.78	100	100	>200	25

TABLE 3. Effect of inoculum size on activity of temocillin

Organism	Concn (μg/ml) required to inhibit 10 isolates at inoculum size (cells/ml) of:					
	10^2		10^4		10^6	
	MIC	MBC	MIC	MBC	MIC	MBC
E. coli	6.25	6.25	12.5	25	12.5	>50
K. pneumoniae	3.12	3.12	6.25	25	6.25	50
P. mirabilis	1.56	12.5	3.12	3.12	6.25	25

and 99% of the isolates at 0.78 μg/ml, respectively. *A. calcoaceticus* and *P. aeruginosa* were highly resistant to temocillin but susceptible to thienamycin and tobramycin.

No significant variation in the MIC of temocillin was observed when the pH was increased from 6.4 to 8.0. Higher concentrations of the antibiotic were required, however, to achieve bactericidal activity at a pH of 8.0. For example, the MIC for 100% inhibition was 6.25 μg/ml for *K. pneumoniae* isolates, whereas the MBC for 100% bactericide was 50 μg/ml at this pH. Medium variation studies in which Trypticase soy broth, brain heart infusion broth, nutrient broth, and Mueller-Hinton broth were used indicated that temocillin was slightly less active in brain heart infusion broth and in nutrient broth. Variations in the inoculum size showed that the antibacterial activity of temocillin against the organisms tested was minimally affected by inoculum sizes within the range of 10^2 to 10^6 organisms per ml (Table 3).

The effect of serum binding on temocillin was minimal. MICs for the 30 isolates of gram-negative bacilli were generally one dilution higher with human serum than the values obtained with Mueller-Hinton broth. For example, temocillin diluted with Mueller-Hinton broth inhibited 100% of the isolates of *E. coli* at a concentration of 6.25 μg/ml, whereas a concentration of 12.5 μg/ml was required to inhibit 100% of the isolates when human serum broth was used as a diluent.

TABLE 4. In vitro activity of temocillin against multiresistant gram-negative bacilli

Organism	No. of isolates tested	MIC (μg/ml)	
		MIC_{75}[a]	Range
Klebsiella spp.	14	3.12	3.12–25
Enterobacter spp.	13	12.5	3.12–12.5
S. marcescens	8	100	3.12–100
Pseudomonas spp.	7	>400	200–>400
Achromobacter spp.	4	>400	25–>400
A. hydrophila	3	>400	1.56–>400
E. coli	2	6.25	3.12–6.25
C. freundii	1	100	100

[a] MIC_{75}, MIC for 75% inhibition.

The in vitro activity of temocillin against multiresistant gram-negative bacilli is summarized in Table 4. Temocillin was active against *E. coli, K. pneumoniae, Enterobacter* spp., two strains of *S. marcescens*, one strain of *A. hydrophila*, and one strain of *Achromobacter* spp. All *P. aeruginosa* isolates and three *Achromobacter* spp. isolates were highly resistant, with MICs exceeding 200 μg/ml. Most isolates of *S. marcescens* were moderately resistant, with MICs of 50 to 100 μg/ml.

DISCUSSION

Temocillin, a new β-lactam antibiotic, inhibited the majority of gram-negative bacilli tested in this study. Our findings confirm the observations of Slocombe et al., who evaluated this compound and found it to be active against isolates of indole-positive *Proteus* spp. and *P. mirabilis, K. pneumoniae, E. coli, Citrobacter* spp., and *Enterobacter* spp. (12). Some isolates of *Citrobacter* spp. in this study were slightly more resistant, which might indicate a higher resistance among clinical isolates from this institution, probably as a consequence of the extensive use of antibiotics in cancer patients.

The activity against indole-positive *Proteus* spp. is comparable to that of tobramycin and thienamycin and higher than that of other cephalosporins such as cefoxitin, cefuroxime, and cefazolin (12). Against *Enterobacter* spp., the activity was higher than that obtained with several "third generation" cephalosporins such as ceftizoxime, ceftazidime, and cefotiam, but comparable to that of moxalactam (1).

A broad difference in the MIC and MBC occurred with various organisms, of which *S. marcescens* is an example. Of these isolates, 76% were inhibited at a concentration of 25 μg/ml. However, the bactericidal activity was minimal at this concentration; only 4% of isolates were killed. The occurrence of this difference has been noted with other β-lactam antibiotics, such as ceftriaxone, and may be clinically important in compromised patients (6).

The lack of activity against gram-positive cocci and *P. aeruginosa* narrows the spectrum of this compound. This is relevant to cancer patients because *P. aeruginosa* frequently causes

infection in this population. Temocillin was inactive against *P. aeruginosa*; this high level of resistance is in contrast to the effectiveness of ceftazidime and thienamycin noted in this study and of piperacillin and cefoperazone noted in a previous study (7).

Infections with gram-positive cocci, particularly *S. aureus*, seem to be increasing in frequency (8). In contrast to temocillin, the newer cephalosporins, e.g., ceftazidime, moxalactam, and ceftizoxime, exhibit significant activity against most gram-positive cocci except enterococci (1). Other penicillin compounds, such as piperacillin, have activity against enterococci and *S. aureus* (9).

The lack of effect on the potency of temocillin by variations in the pH of the medium is in agreement with observations made in the in vitro evaluations of new penicillins and cephalosporins (7, 14).

The observation that the antibacterial activity of temocillin was not affected by variations in the inoculum size from 10^2 to 10^6 organisms per ml is in agreement with the findings of Slocombe et al. (12).

Pharmacology studies have demonstrated that after a single intravenous dose of 1 g the mean peak serum level of temocillin is 172 ± 33.1 μg/ml and that levels in excess of 25 μg/ml are detected for up to 6 h (12). The MICs for 33 of 52 (63%) isolates of multiresistant organisms were within the ranges of concentrations that can be readily achieved in serum. Furthermore, eight additional isolates had MICs from 50 to 100 μg/ml, which at higher doses of the antibiotic given at 4-h intervals could conceivably be eventually eradicated if they were causing infection.

The spectrum of activity of temocillin and its effect upon resistant organisms, stability against β-lactamases, and favorable pharmacokinetic characteristics indicate that this is a potentially useful therapeutic agent for most gram-negative bacillary infections.

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