IN VITRO AND *IN VIVO* ACTIVITY OF PIPERACILLIN, A NEW BROAD-SPECTRUM SEMISYNTHETIC PENICILLIN

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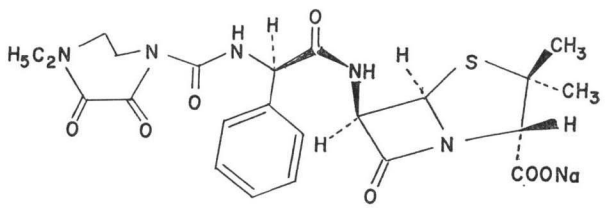
Piperacillin sodium is a novel semisynthetic penicillin with broad spectrum activity against Gram-negative and -positive bacteria including anaerobes. In agar dilution tests with 522 clinical isolates, it inhibited at least 70~90% of each group of organisms at 16 mcg/ml with the exception of the *Serratia* group which required 64 mcg/ml to inhibit 70% of the isolates. Piperacillin was more potent than carbenicillin, ticarcillin and ampicillin against *Klebsiella*, *Pseudomonas, Enterobacter* and *Serratia*. Against Gram-positive cocci it was generally comparable to carbenicillin and ticarcillin except in the case of enterococci where it was significantly more potent. Piperacillin exhibited a broader spectrum than the cephalosporins which lacked antipseudomonal activity. It was more potent than cephalothin, cefamandole and cefoxitin against indole-positive *Proteus, Acinetobacter* and enterococci. Piperacillin is bactericidal; minimal bactericidal concentrations were within four-fold of the minimal inhibitory concentrations. Its activity was not significantly altered by pH, media or serum, but was reduced when inoculum size was increased from 10^5 to 10^7 CFU/ml. Synergistic effects were observed when piperacillin was combined with gentamicin or amikacin. Piperacillin was effective against both acute lethal infections and chronic kidney infections in mice. Our results indicate that piperacillin has a spectrum of activity that spans those of other semisynthetic penicillins and cephalosporins.

Piperacillin sodium, sodium[2*S*-[2α, 5α, 6β(S*)]]-6-[[[[(4-ethyl-2,3-dioxo-1-piperazinyl)carbonyl]-amino]phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate, originally referred to as T-1220, is a new semisynthetic penicillin developed by Toyama Chemical Company, Ltd. (Fig. 1). Laboratory studies showing its broad spectrum of activity against Gram-negative and -positive bacteria were first reported by MITSUHASHI, SAIKAWA and YASUDA in 1976 at the 16th Interscience Conference on Antimicrobial Agents & Chemotherapy. Its superior potency over other semisynthetic penicillins against Gram-negative bacteria has been reported by several investigators[1~6]. This report deals with a comparison of the *in vitro* activities of piperacillin sodium with other penicillins and with cephalosporins against 522 clinical isolates and with its effect against experimental infections in mice.

Fig. 1. Piperacillin sodium



Materials and Methods

Antibiotics

Piperacillin sodium was obtained from Toyama Chemical Company, Ltd. Agents used for comparison were carbenicillin disodium (Roerig-Pfizer); ticarcillin disodium and ampicillin (Beecham

Pharmaceuticals); cephalothin sodium and cefamandole nafate (Eli Lilly & Co.); and cefoxitin (Merck & Co.). Gentamicin sulfate (Schering Corp.) and amikacin (Bristol Laboratories) were used in synergism tests.

Test strains

Clinical isolates were obtained from 14 different medical centers representing various geographical locations in the United States.

In vitro Minimal inhibitory concentrations (MICs) of agents were determined by means of standard two-fold serial dilution methods using agar or broth media. In most cases MUELLER-HINTON broth or agar was used. Streptococci were tested in M-H agar supplemented with 5% sheep blood; *Haemophilus* cultures were tested in Brain Heart Infusion agar supplemented with FILDES enrichment; anaerobes were tested in *Brucella* agar supplemented with 5% sheep blood and menadione; *Proteus mirabilis* cultures were tested in Nutrient agar to avoid swarming. In the agar dilution tests, the agar surfaces were inoculated with approximately 10^4 colony forming units (CFU) of each strain by means of the Steers multiple inocula replicator. The lowest concentration of drug that inhibited macroscopic growth of a culture after 20 hours incubation at 37°C was recorded as the MIC. The microtiter system was used for the broth dilution tests. Volumes of 0.05 ml antibiotic solutions in broth were inoculated with 0.05 ml of a broth dilution of a 5-hour culture. The inoculum was approximately 10^5 CFU/ml. The lowest concentration that inhibited visible growth after 20 hours at 37°C incubation was recorded as the MIC. Immediately after the determination of the MICs, 0.01 ml samples were removed from the inhibited cultures and plated on agar. The lowest concentration of antibiotic associated with no growth after overnight incubation of the plate ($\geq$99.9% kill) was recorded as the minimal bactericidal concentration (MBC).

The effect of inoculum size and serum were determined in broth dilution tests. Media, pH and synergistic effects were determined in agar dilution tests.

In vivo The protective effects of antibiotics were assessed in 18~20 g female mice, strain CD-1 (Charles River Laboratories) or Strain CF-1 (Carworth Farms).

Acute lethal infections were produced by the intraperitoneal injection of sufficient organisms suspended in 0.5 ml of broth or mucin to kill 90~100% of nontreated mice within 48 hours. Antibiotics were administered by the subcutaneous route approximately 30 minutes after infection and in some cases a second dose was administered 3 or 4 hours later. In a test, 5~10 mice were tested at each dose level. Survival ratios were determined 7~14 days after infection. The results of 2~4 separate tests were pooled for the determination of median effective doses (ED_{50}) by probit analysis.

Chronic kidney infections were induced by the intravenous injection of bacteria suspended in 0.2 ml of broth. In the case of the enterococcal infection, treatment was administered 3, 18, 22 and 26 hours after infection. Mice were sacrificed for the examination of kidneys 72 hours after the last treatment. In the case of the *Proteus mirabilis* kidney infection, treatment was administered 3 hours after infection and twice daily for 6 days. Mice were sacrificed 24 hours after the last treatment. The effectiveness of the antibiotics was assessed by examining the kidneys for the presence of the infecting organisms. Each kidney was macerated and spread on agar plates which were incubated at 37°C for 24 hours. Kidneys from nontreated infected mice yielded confluent growth while those from noninfected mice showed no growth. Treatment was considered protective if the growth was less than 50 colonies per kidney.

Results

In Vitro

Piperacillin sodium demonstrated a broader spectrum of activity and in several cases greater potency than did carbenicillin, ticarcillin, ampicillin, or the cephalosporin antibiotics cephalothin, cefamandole or cefoxitin (Table 1). At 16 mcg/ml, piperacillin sodium inhibited at least 70% of the strains in each group of organisms tested with the exception of *Serratia*; 64 mcg/ml of piperacillin was required to

Table 1. *In vitro* activity of piperacillin sodium compared with penicillins and cephalosporins*

Organism		% strains inhibited	Concentration, mcg/ml, required to inhibit indicated % of strains						
			Piperacillin	Carbenicillin	Ticarcillin	Ampicillin	Cephalothin	Cefamandole	Cefoxitin
Pseudomonas	(60)**	50	2	64	16				
		70	4	64	32				
		90	8	128	32				
Klebsiella	(62)	50	4	>128	128	64	4	1	2
		70	16		>128	128	4	2	4
		90	>128			>128	32	8	4
Enterobacter	(50)	50	4	16	4	128	>128	2	>128
		70	8	64	64	>128		16	
		90	128	>128	>128			128	
Serratia	(29)	50	16	>128	>128	>128	>128	64	16
		70	64					128	16
		90	128					>128	64
Proteus indole-negative	(50)	50	0.5	1	1	0.5	1	1	2
		70	0.5	1	1	0.5	1	1	2
		90	0.5	2	2	0.5	2	1	2
Proteus indole-positive	(29)	50	1	2	2	>128	>128	8	8
		70	4	32	16			16	16
		90	128	>128	>128			32	16
Escherichia coli	(62)	50	2	4	2	4	8	0.5	4
		70	2	8	8	8	16	1	4
		90	>128	>128	>128	>128	32	8	8
Salmonella	(31)	50	2	4	2	2	2	0.5	2
		70	2	8	4	2	4	0.5	4
		90	4	8	4	4	8	2	4
Acinetobacter	(31)	50	8	8	8	16	>128	32	32
		70	16	8	8	32		32	64
		90	16	16	16	32		64	64
Haemophilus influenzae	(15)	50	0.5	0.5	0.5	0.5	nt	2	8
		70	0.5	0.5	0.5	0.5		2	8
		90	0.5	0.5	0.5	0.5		32	16
Bacteroides fragilis	(11)	50	4	16	32	nt	nt	64	8
		70	4	16	32			64	16
		90	4	16	32			64	16
Enterococcus	(31)	50	2	32	64	1	32	32	>128
		70	2	32	64	1	32	32	
		90	4	32	64	1	32	32	
Streptococcus pyogenes	(16)	50	<0.5	0.12	0.25	<0.5	<0.5	<0.5	0.5
		70	<0.5	0.12	0.25	<0.5	<0.5	<0.5	0.5
		90	<0.5	0.12	0.25	<0.5	<0.5	<0.5	0.5
Streptococcus pneumoniae	(15)	50	<0.5	<0.5	<0.5	<0.5	0.12	0.12	0.5
		70	<0.5	<0.5	<0.5	<0.5	0.12	0.12	1
		90	<0.5	0.5	0.5	<0.5	0.12	0.12	2
Staphylococcus aureus	(30)	50	2	4	4	1	<0.5	1	4
		70	8	8	8	4	0.5	1	4
		90	128	32	32	32	1	2	16

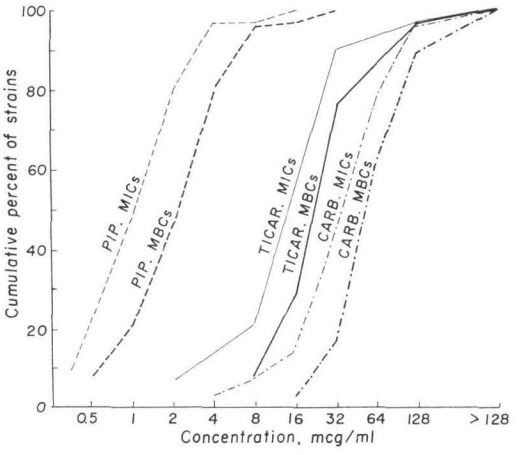
* Agar dilution method

** Figures in parentheses indicate number of strains tested.

inhibit 70% of *Serratia*. The other semisynthetic penicillins and the cephalosporin antibiotics required greater concentrations to inhibit 70% of seveal types of organisms.

Piperacillin sodium was remarkably active against several groups of Gram-negative bacteria, in particular, *Pseudomonas, Klebsiella, Enterobacter,* and *Serratia.* Against these organisms it was more potent than carbenicillin, ticarcillin and ampicillin. Of special note is its potency against *Pseudomonas*; the MBCs of piperacillin were less than the MICs of carbenicillin and ticarcillin (Fig. 2). The cephalosporin antibiotics were more potent than piperacillin sodium against *Klebsiella,* but against *Enterobacter* piperacillin sodium was significantly more potent than cefoxitin and cephalothin and similar to cefamandole. Piperacillin sodium was more potent than cefamandole and cephalothin against *Serratia* while cefoxitin was more potent than piperacillin against some of the strains. Against indole-positive *Proteus,* piperacillin sodium was more potent than the cephalosporin antibiotics. It was also more potent than the cephalosporins against *Acinetobacter,* but had no advantage over carbenicillin or ticarcillin. Piperacillin sodium was active against strains of *Escherichia coli, Proteus mirabilis* and *Haemophilus influenzae* but demonstrated no significant superiority.

Fig. 2. Inhibitory and bactericidal effects of piperacillin sodium *vs. Pseudomonas.*



Piperacillin sodium was active against *Bacteroides fragilis* and exceeded carbenicillin, ticarcillin, cefamandole and cefoxitin in potency.

Piperacillin sodium was active against Gram-positive bacteria. It was generally comparable to carbenicillin or ticarcillin against strains of *Staphylococcus aureus, Streptococcus pyogenes* and *Streptococcus pneumoniae.* Against strains of enterococcus, however, piperacillin sodium was significantly more potent than carbenicillin, ticarcillin and the cephalosporins.

Table 2. Synergistic effects of piperacillin sodium combined with gentamicin or amikacin (Agar dilution method - MUELLER-HINTON agar)

Organism	Minimal inhibitory concentrations, mcg/ml				
	Piperacillin alone	Gentamicin alone	Amikacin alone	Piperacillin + gentamicin	Piperacillin + amikacin
Pseudomonas aeruginosa					
OSU-75-1	2	8	8	0.5+2	0.5+2
MA-75-1	16	16	16	0.5+4	1 +4
Enterobacter sp. B-10014	16	1	2	2 +0.25	4 +0.5
Serratia marcescens SL-445	128	1	2	8 +0.25	8 +0.5
K-77A	128	1	4	8 +0.25	2 +1
					16 +0.25
Acinetobacter calcoaceticus					
AEMC-75-5	16	4	2	2 +1	4 +0.5
Enterococcus (*S. faecalis*)					
KC-74-2	4	16	>128	1 +4	indifferent
OSU-75-2	4	16	>128	1 +4	indifferent

Table 3. Protective effects of piperacillin sodium against experimental infections in mice

Infection: Organism	Approx. CFU/mouse (diluent)	Treatment sc hours after infection	Piperacillin		Carbenicillin		Ticarcillin		Ampicillin	
			MIC	ED_{50}*	MIC	ED_{50}	MIC	ED_{50}	MIC	ED_{50}
Klebsiella pneumoniae AD	7.1×10^4 (broth)	1/2	0.5	28 (20~39)	8	110 (89~150)	2	100 (75~140)	0.5	2.4 (1.6~3.3)
〃 〃 W–75–2	6.0×10^4 (2.5% mucin)	1/2	8	18 (15~23)	>128	>256	>128	>256	64	50 (41~60)
〃 〃 124–13	2.0×10^3 (broth)	1/2	8	230 (150~370)	>128	>512	>128	>512	128	>512
Enterobacter aerogenes #75	1.1×10^6 (5% mucin)	1/2	4	27 (21~35)	>128	210 (180~240)	>128	190 (160~240)	32	38 (31~45)
Serratia marcescens F–35	6.0×10^4 (5% mucin)	1/2, 3	8	4.5 (3.8~5.2)	>128	>256	>128	>256	>128	>256
Pseudomonas aeruginosa 12–4–4	7.5×10^3 (2.5% mucin)	1/2, 4	4	17 (10~24)	64	50 (35~71)	16	32 (25~40)	>128	>256
〃 〃 PD 05141	5.6×10^4 (5% mucin)	1/2, 4	2	22 (17~28)	64	57 (46~69)	16	34 (28~43)	>128	130 (97~170)
〃 〃 G236	4.2×10^6 (5% mucin)	1/2, 4	4	30 (18~43)	32	50 (41~62)	16	62 (47~83)	>128	>256
Escherichia coli #311	1.0×10^4 (broth)	1/2	1	5.5 (4.6~6.4)	4	7.3 (6.0~8.8)	4	12 Estimate	4	4.5 (3.5~5.6)
Proteus mirabilis OSU–75–1	1.6×10^4 (5% mucin)	1/2	0.5	3.1 (2.6~3.8)	1	9.8 (8.0~12)	1	9.8 (8.3~12)	2	2.9 (2.5~3.5)
Proteus rettgeri N–74–1	1.8×10^3 (5% mucin)	1/2	1	12 (7~22)	2	7.9 (4.1~15)	2	6.5 (2.9~14)	64	180 (130~250)
Staphylococcus aureus Smith	7.2×10^6 (broth)	1/2	1	4.2 (3.1~5.7)	1	1.7 (1.2~2.3)	1	2.9 (2.2~4.2)	0.12	0.8 (0.6~1.1)

* ED_{50}=mg/kg/sc dose. Figures in parentheses are 95% confidence limits.

Piperacillin is bactericidal. The MBCs were generally equal to or only 2- to 4-fold higher than the MICs for a variety of Gram-positive and -negative strains including *Pseudomonas* (Fig. 2). The bactericidal effect was rapid. The minimal inhibitory concentration of piperacillin reduced an inoculum of 10^5 CFU/ml of a *Pseudomonas* culture 99.9% in 6 hours. In the case of a *Klebsiella* culture twice the MIC reduced the inoculum 99% in 6 hours and to undetectable levels in 24 hours.

The efficacy of piperacillin and carbenicillin was not influenced by medium, pH (6.0~7.5) or 50% human serum. The efficacy of piperacillin, as well as that of carbenicillin and ticarcillin, was affected by the size of the inoculum. An increase in inoculum size from 10^3 to 10^5 CFU/ml of representative strains of *Pseudomonas, Klebsiella, Enterobacter, Proteus, Staphylococcus* and *Enterococcus* was without significant effect but MICs and particularly MBCs were elevated 4~>64-fold when the inoculum was raised from 10^5 to 10^7 CFU/ml.

Synergistic effects against a variety of organisms were observed when piperacillin sodium was combined with gentamicin or amikacin (Table 2). The combination of drugs was considered synergistic when the MIC of both drugs was reduced at least four-fold. In these tests the MICs of the aminoglycoside antibiotics were generally not reduced more than four-fold while those of piperacillin sodium were reduced 4- to 32-fold. No antagonism was observed. The enterococcal strains were highly resistant to amikacin and no synergistic effects were observed when this aminoglycoside antibiotic was combined with piperacillin sodium. These strains were less resistant to gentamicin and synergism was effected by the combination of gentamicin and piperacillin.

In Vivo

Piperacillin sodium was effective in protecting mice from lethal infections produced by *in vitro* susceptible organisms (Table 3). It was highly effective against infections produced by strains of *Klebsiella, Enterobacter* and *Serratia* including those that were resistant to carbenicillin, ticarcillin, or ampicillin. Against *Pseudomonas* infections piperacillin, on a dosage basis, was 1.5~3 times more potent than carbenicillin and ticarcillin, and considerably more potent than ampicillin. Relatively low doses of all the penicillins protected against lethal infections produced by strains of *E. coli, Proteus* and *Staphylococcus*.

Piperacillin sodium was also effective in protecting mice against chronic kidney infections (Table 4). It protected mice against a *Proteus mirabilis* kidney infection at doses approximately 4-fold lower than

Table 4. Protective effects of piperacillin against kidney infections

	Treatment subcutaneous mg/kg/dose	Protection ratios[a]			
		Piperacillin	Carbenicillin	Ticarcillin	Ampicillin
Proteus mirabilis OSU-75-1[b]	512	10/10	10/10	10/10	10/10
	128	10/10	5/10	5/10	5/10
	32	5/10	0/10	0/10	0/10
	8	0/10	—	—	—
Enterococcus SM-77-1[c]	128	10/10	4/10		
	32	10/10	0/10		
	8	1/10	0/10		

[a] Protection ratio: No. mice protected/No. mice tested. Pooled data from 2 tests of each infection.
[b] Treatment: 3 hours after infection and twice daily for 6 days.
[c] Treatment: 3, 18, 22 and 26 hours after infection.

those of carbenicillin, ticarcillin, or ampicillin. The superiority of piperacillin sodium over carbenicillin against enterococcus shown *in vitro* was demonstrated *in vivo*. Mice were protected from an enterococcal kidney infection by doses of piperacillin sodium that were ineffective with carbenicillin.

Discussion

Our studies show that piperacillin sodium has a broad spectrum of activity against both Gram-negative and -positive bacteria. Its spectrum spans those of other semisynthetic penicillins and the cephalosporins. It is active against clinical isolates susceptible to carbenicillin, ticarcillin or ampicillin, but in addition has greater potency particularly against *Pseudomonas, Klebsiella, Enterobacter, Serratia* and enterococcus. Piperacillin sodium is active against most bacteria that are susceptible to the cephalosporins and, in addition, has activity against *Pseudomonas*. It also has greater potency than the cephalosporins against *Acinetobacter* and enterococcal strains.

Our results are in general agreement with other investigators. UEO *et al.*[1] reported that piperacillin was more active than carbenicillin particularly against *Klebsiella, Pseudomonas, Proteus* spp. and *Serratia*. VERBIST[2] reported that piperacillin combines the spectrum of ampicillin and carbenicillin but was generally superior because of its intrinsically higher potency. He makes special note of the good activity of piperacillin against *Klebsiella, Enterobacter, Citrobacter* and *Pseudomonas*. Although reporting somewhat higher MIC values, FU and NEU[3] also showed the general superiority of piperacillin over carbenicillin, ticarcillin and ampicillin. WINSTON *et al.*[4] tested 3,600 facultative Gram-negative bacilli, *Bacteroides* and enterococcus and found that, except for *E. coli*, piperacillin inhibited 90% or more of isolates in each group of organisms at $\leq$64 mcg/ml. Further, they note that compared to carbenicillin, ampicillin, cephalothin, cefoxitin and gentamicin, piperacillin has superior therapeutic indices (ratio between the mean peak clinical serum level of antibiotic and the MIC required to inhibit two-thirds of the strains in each group of organisms). JONES *et al.*[5] reported that in their tests 91% of 394 clinical isolates of Enterobacteriaceae, *Pseudomonas* and *Staphylococcus* were inhibited by 16 mcg/ml of piperacillin.

Piperacillin sodium is bactericidal. In our tests with a standard inoculum of 10^5 CFU/ml, the MBCs were within 4-fold of the MICs for various types of organisms. Thus we are in agreement with UEO *et al.* and VERBIST, and for most strains we are also in agreement with WINSTON *et al.* and FU and NEU. These latter investigators, however, found greater differences between MICs and MBCs for certain strains of *Pseudomonas*. All investigators report that like other penicillins, piperacillin activity is influenced by inoculum size.

Although we tested only a few strains, we show that piperacillin is capable of synergistic effects when combined with gentamicin or amikacin. Using large numbers of strains, FU and NEU demonstrated synergistic effects of piperacillin and aminoglycosides against a variety of strains and note particularly the excellent synergy of piperacillin and amikacin against *Serratia*. WINSTON *et al.* observed excellent synergy with piperacillin and amikacin, particularly against *Pseudomonas, Klebsiella* and *Serratia*.

Piperacillin is effective against both lethal and chronic bacterial infections in mice. It was generally more effective on a dosage basis than carbenicillin, ticarcillin or ampicillin. UEO *et al.* also demonstrated the greater *in vivo* effectiveness of piperacillin compared to carbenicillin and ampicillin against *Klebsiella* and *Pseudomonas* infections.

Our laboratory studies, and those of other investigators, indicate that because of its broad spectrum of activity and potency, piperacillin sodium merits further investigation to determine its clinical potential.

References

1) UEO, K.; Y. FUKUOKA, T. HAYASHI, T. YASUDA, H. TAKI, M. TAI, Y. WATANABE, I. SAIKAWA & S. MITSUHASHI: *In vitro and in vivo* antibacterial activity of T-1220, a new semisynthetic penicillin. Antimicr. Agents & Chemoth. 12: 455~460, 1977

2) VERBIST, L.: *In vitro* activity of piperacillin, a new semisynthetic penicillin with an unusually broad spectrum of activity. Antimicr. Agents & Chemoth. 13: 349~357, 1978
3) FU, K. P. & H. C. NEU: Piperacillin, a new penicillin active against many bacteria resistant to other penicillins. Antimicr. Agents & Chemoth. 13: 358~367, 1978
4) WINSTON, D. J.; D. WANG, L. S. YOUNG, W. J. MARTIN & W. L. HEWITT: *In vitro* studies of piperacillin, a new semisynthetic penicillin. Antimicr. Agents & Chemoth. 13: 944~950, 1978
5) JONES, R. N.; C. THORNSBERRY, A. L. BARRY, P. C. FUCHS, T. L. GAVAN & E. H. GERLACH: Piperacillin (T-1220), a new semisynthetic penicillin: *In vitro* antimicrobial activity comparison with carbenicillin, ticarcillin, ampicillin, cephalothin, cefamandole and cefoxitin. J. Antibiotics 30: 1107~1114, 1977
6) RENKONEN, O-V.: Susceptibility of 500 clinical isolates to piperacillin. *In* Current Chemotherapy Proceedings of the 10th International Congress of Chemotherapy, p. 601. Amer. Soc. Microb., Washington, D.C., 1978