ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 1989, p. 1964–1969
0066-4804/89/111964-06$02.00/0

Vol. 33, No. 11

Comparative In Vitro and In Vivo Activities of Piperacillin Combined with the β-Lactamase Inhibitors Tazobactam, Clavulanic Acid, and Sulbactam

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Received 20 March 1989/Accepted 1 August 1989

Tazobactam (YTR-830H), a novel β-lactamase inhibitor, was compared with clavulanic acid and sulbactam for enhancement of the activity of piperacillin against β-lactamase-producing, piperacillin-resistant clinical isolates. Piperacillin MICs were determined in media containing a fixed concentration of 2 or 4 μg of the inhibitors per ml. The higher concentration was generally more effective. Tazobactam was superior to sulbactam in enhancing the spectrum and potency of piperacillin. Although the clavulanic acid combination was more potent, tazobactam was effective for a similar spectrum of resistant gram-negative clinical isolates containing β-lactamase. MICs were reduced to the susceptible range for *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus* spp., *Salmonella* spp., and *Shigella* spp. Combinations with tazobactam and sulbactam, but not clavulanic acid, were effective against *Morganella* spp. Some antagonism of the activity of piperacillin was observed with clavulanic acid but not with tazobactam or sulbactam. The inhibitors were similarly effective with piperacillin against β-lactamase-positive *Staphylococcus* spp. and the *Bacteroides fragilis* group. Piperacillin-tazobactam was more effective against a broader spectrum of gram-negative enteric bacteria than ticarcillin plus clavulanic acid was. Combinations with tazobactam or clavulanic acid had a broader spectrum of activity than combinations with sulbactam against bacteria that produce characterized plasmid-mediated enzymes of clinical significance. In particular, piperacillin with tazobactam or clavulanic acid, but not with sulbactam, inhibited TEM-1, TEM-2, and SHV-1 enzymes. In vitro activity was reflected in vivo. Tazobactam and clavulanic acid were superior to sulbactam in enhancing the therapeutic efficacy of piperacillin in mice infected with β-lactamase-positive *E. coli*, *K. pneumoniae*, *Proteus mirabilis*, and *Staphylococcus aureus*. Only combinations with tazobactam and sulbactam were effective against the *Morganella* infection. Tazobactam has a good potential for enhancing the clinical efficacy of piperacillin.

Tazobactam (YTR-830H) is a novel triazolymethyl penicillanic acid sulfone β-lactamase inhibitor that acts on a variety of clinically important β-lactamases. Piperacillin is a potent broad-spectrum antibiotic but is susceptible to some β-lactamases. Investigators have shown that tazobactam can enhance the activities of β-lactam antibiotics, including piperacillin (1; N. Ishida, A. Hyodo, C. Hanehara, Y. Miyake, Y. Kawaguchi, and J. Tamabe, Proc. 14th Int. Congr. Chemother., p. 1274–1275, 1985). In a previous study we showed that piperacillin-tazobactam combinations in ratios of 4:1 and 8:1 reduced the MICs of piperacillin from the resistant to the susceptible or moderately susceptible range for many β-lactamase-producing isolates of enteric bacteria, *Staphylococcus* spp., and *Bacteroides* spp. (N. A. Kuck, P. J. Petersen, W. J. Weiss, N. V. Jacobus, R. T. Testa, and F. P. Tally, Program Abstr. 6th Mediterr. Cong. Chemother., abstr. no. 155, 1988). In the present study we compared fixed concentrations of tazobactam with sulbactam and clavulanic acid for effects on the in vitro and in vivo activity of piperacillin against clinical isolates of bacteria and laboratory strains that produce specific plasmid-mediated β-lactamases. Piperacillin-inhibitor combinations in ratios of 4:1 and 8:1 were used to assess protective responses in mice infected with piperacillin-resistant bacteria.

(A part of this study was presented at the 28th Interscience Conference on Antimicrobial Agents and Chemotherapy, Los Angeles, Calif., 23 to 26 October 1988 [N. V. Jacobus, W. Weiss, P. Petersen, N. A. Kuck, and R. T. Testa, Program Abstr. 28th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 108, 1988].)

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MATERIALS AND METHODS

Bacterial isolates were collected during 1987 and 1988 from medical centers located in various geographical areas. Cultures were stored frozen in skim milk at −70°C. Identification of each culture was confirmed by conventional procedures: gram-negative rods and *Staphylococcus* spp. by API systems and anaerobes by the procedures of Holdeman et al. (2, 4, 7). β-Lactamase production was determined by a nitrocefin disk (Cefinase; BBL Microbiology Systems). Selection of gram-negative resistant strains was based on piperacillin MICs of ≥128 μg/ml. Susceptibility of staphylococci to oxacillin was determined by the presence or absence of growth on an agar plate containing 6 μg of oxacillin per ml, as described by Thornsberry and McDougal (12). Cultures that produce characterized plasmid-mediated β-lactamases were kindly provided by A. A. Medeiros, Brown University, Providence, R.I. The plasmids and β-lactamases from these strains have been isolated and characterized (8). The agents used in the tests were piperacillin and tazobactam (Lederle Laboratories), clavulanic acid and ticarcillin (Beecham Laboratories), and sulbactam (Pfizer Inc.).

In vitro. Susceptibility tests were performed in broth or agar with a fixed concentration of 2 or 4 μg of an enzyme inhibitor per ml and twofold serial dilutions of piperacillin or ticarcillin. MICs for enteric gram-negative bacteria and

TABLE 1. Comparison of tazobactam, clavulanic acid, and sulbactam for enhancement of piperacillin activity against piperacillin-resistant, β-lactamase-producing enteric bacteria; comparison with ticarcillin plus clavulanic acid

Organism (no. of strains tested)	Agent[a]	MIC (μg/ml)[b]		
		Range	50%	90%
Escherichia coli (30)	PIP +			
	TZB 2	1–>128	2	128
	TZB 4	0.5–>128	2	32
	CVA 2	1–>128	2	4
	CVA 4	0.5–>128	1	4
	SBT 2	8–>128	128	>128
	SBT 4	2–>128	64	>128
	TIC +			
	CVA 2	16–>128	64	128
	TZB	128–>128	128	>128
	CVA	16–64	16	32
	SBT	16–>128	32	64
Klebsiella pneumoniae (16)	PIP +			
	TZB 2	1–32	4	32
	TZB 4	1–8	4	8
	CVA 2	1–8	2	8
	CVA 4	1–16	4	8
	SBT 2	1–>128	32	>128
	SBT 4	1–>128	32	128
	TIC +			
	CVA 2	2–>128	32	>128
	TZB	128–>128	>128	>128
	CVA	16–64	32	32
	SBT	32–128	32	64
Klebsiella oxytoca (12)	PIP +			
	TZB 2	1–>128	4	>128
	TZB 4	1–>128	2	>128
	CVA 2	2–64	4	8
	CVA 4	1–64	2	8
	SBT 2	4–>128	64	>128
	SBT 4	2–>128	32	>128
	TIC +			
	CVA 2	8–>128	64	>128
	TZB	64–>128	128	>128
	CVA	32	32	32
	SBT	32–>128	64	128
Enterobacter cloacae (12)	PIP +			
	TZB 2	4–>128	64	128
	TZB 4	2–>128	32	128
	CVA 2	2–>128	128	128
	CVA 4	2–>128	128	>128
	SBT 2	32–>128	128	>128
	SBT 4	32–>128	128	>128
	TIC +			
	CVA 2	32–>128	>128	>128
	TZB	128	>128	>128
	CVA	32–64	64	64
	SBT	>128	64	128
Enterobacter aerogenes (10)	PIP +			
	TZB 2	2–>128	32	>128
	TZB 4	2–>128	16	>128
	CVA 2	2–>128	32	>128
	CVA 4	2–>128	32	>128
	SBT 2	32–>128	64	>128
	SBT 4	16–>128	32	>128
	TIC +			
	CVA 2	32–>128	128	>128
	TZB	128–>128	>128	>128
	CVA	16–64	16	64
	SBT	64–>128	64	128
Serratia spp. (13)	PIP +			
	TZB 2	0.5–128	64	128
	TZB 4	0.5–128	32	128
	CVA 2	1–128	64	128
	CVA 4	1–>128	64	>128
	SBT 2	4–>128	128	128
	SBT 4	1–>128	64	128
	TIC +			
	CVA 2	16–>128	128	>128
	TZB	128–>128	>128	>128
	CVA	64–128	64	128
	SBT	64–>128	128	128
Proteus mirabilis (10)	PIP +			
	TZB 2	0.25	0.25	0.25
	TZB 4	0.25–0.5	0.25	0.5
	CVA 2	0.25–0.5	0.25	0.5
	CVA 4	0.25–0.5	0.25	0.5
	SBT 2	0.5–16	2	16
	SBT 4	0.25–8	0.5	0.5
	TIC +			
	CVA 2	1–8	8	8
	TZB	>128	>128	>128
	CVA	64–128	64	128
	SBT	128–>128	128	>128
Morganella morganii (10)	PIP +			
	TZB 2	0.5–16	2	16
	TZB 4	0.12–8	0.5	8
	CVA 2	64–>128	128	>128
	CVA 4	64–>128	128	>128
	SBT 2	4–128	16	128
	SBT 4	1–64	4	64
	TIC +			
	CVA 2	8–>128	32	32
	TZB	>128	>128	>128
	CVA	64–>128	128	128
	SBT	128–>128	128	>128
Providencia spp. and *Proteus* (indole +) spp. (14)	PIP +			
	TZB 2	0.12–>128	2	128
	TZB 4	0.12–128	2	2
	CVA 2	0.25–32	2	16
	CVA 4	0.25–16	2	16
	SBT 2	0.25–>128	4	>128
	SBT 4	0.25–>128	4	>128
	TIC +			
	CVA 2	0.5–128	8	32
	TZB	128–>128	>128	>128
	CVA	64–128	64	128
	SBT	64–128	128	128
Citrobacter diversus (10)	PIP +			
	TZB 2	2–>128	16	>128
	TZB 4	2–>128	4	>128
	CVA 2	2–32	8	32
	CVA 4	2–64	4	64
	SBT 2	64–>128	128	>128
	SBT 4	64–>128	128	>128
	TIC +			
	CVA 2	128–>128	>128	>128
	TZB	128–>128	>128	>128
	CVA	16–64	32	64
	SBT	64–>128	128	>128

Continued

TABLE 1—*Continued*

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TABLE 1—*Continued*

Organism (no. of strains tested)	Agent[a]	MIC (μg/ml)[b]		
		Range	50%	90%
Citrobacter freundii (10)	PIP +			
	TZB 2	2–>128	128	>128
	TZB 4	1–128	64	128
	CVA 2	2–>128	128	>128
	CVA 4	2–>128	64	>128
	SBT 2	32–>128	128	>128
	SBT 4	16–>128	128	>128
	TIC +			
	CVA 2	16–>128	>128	>128
	TZB	128–>128	>128	>128
	CVA	32–64	32	64
	SBT	32–128	64	128
Salmonella spp. and *Shigella* spp. (17)	PIP +			
	TZB 2	0.5–>128	4	32
	TZB 4	0.25–>128	2	4
	CVA 2	0.5–8	2	8
	CVA 4	0.25–4	2	4
	SBT 2	4–>128	64	>128
	SBT 4	2–>128	128	>128
	TIC +			
	CVA 2	16–>128	128	>128
	TZB	128–>128	128	>128
	CVA	16–128	32	64
	SBT	32–>128	64	128

[a] Antibiotics were tested with a fixed concentration of 2 or 4 μg of inhibitor per ml. Abbreviations: PIP, piperacillin; TZB, tazobactam; CVA, clavulanic acid; SBT, sulbactam; TIC, ticarcillin.
[b] In combinations, MICs are in terms of piperacillin or ticarcillin.

staphylococci were determined by the microdilution method in 0.1-ml cation-supplemented Mueller-Hinton broth (BBL) according to National Committee for Clinical Laboratory Standards recommendations (10). The final inoculum density was 5×10^5 CFU/ml. The microdilution panels were incubated at 35°C for 18 to 20 h. Anaerobic bacteria were tested in Wilkins-Chalgren agar supplemented with 5% lysed sheep blood. The inoculum was 1×10^5 to 5×10^5 CFU per spot, applied with a Steers replicator. Incubation was at 37°C for 48 h in an anaerobic chamber. Recommended American Type Culture Collection quality control cultures were included in all in vitro tests to assure acceptable responses to piperacillin or ticarcillin (10, 11).

In vivo. Therapeutic effects were assessed in mice infected with bacteria that produce β-lactamase and were resistant to piperacillin. Female CD-1 mice, 20 ± 2 g, from Charles River Breeding Laboratories were challenged by the intraperitoneal injection of bacterial suspensions in 0.5 ml of hog gastric mucin (10 to 200 50% lethal doses). Four or five dose levels of piperacillin and the β-lactamase inhibitors as single agents or in combinations of 4:1 or 8:1 were prepared in 0.2% aqueous agar. Each dose preparation was administered subcutaneously to five mice 0.5 h postinfection. With the *Morganella* spp. infection, a second dose was injected 3 h later. All the untreated control animals died within 48 h of infection. Median (50%) effective dose were determined by probit analysis of the 7-day survival ratios pooled from three or four separate tests.

RESULTS

In vitro. Tazobactam enhanced the activity of piperacillin against a broad spectrum of piperacillin-resistant β-lactamase-producing clinical isolates. Among the enteric isolates, the combination was particularly effective in reducing the MICs of piperacillin from the resistant (≥128 μg/ml) to the susceptible (≤16 μg/ml) or moderately susceptible (32 to 64 μg/ml) range for *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus* spp., *Providencia* spp., *Morganella* spp., *Salmonella* spp., and *Shigella* spp. (Table 1). Clavulanic acid, the most active of the inhibitors when tested as a single agent, reduced the MICs of piperacillin 2- to 16-fold lower than tazobactam for most bacteria, except for *Morganella* spp. Unlike tazobactam and sulbactam, clavulanic acid did not enhance the activity of piperacillin against *Morganella* spp. Tazobactam and clavulanic acid were equally effective in reducing the MICs of piperacillin against *Proteus mirabilis*. Sulbactam reduced the piperacillin MICs for 90% of strains (MIC_{90}) to the susceptible range only against *Proteus* spp. and *Morganella* spp. The MICs of piperacillin were generally lower when piperacillin was combined with 4 μg than when it was combined with 2 μg of tazobactam per ml. Piperacillin combined with either 2 or 4 μg of tazobactam per ml showed a broader spectrum of activity than ticarcillin combined with clavulanic acid. Susceptible or intermediate MIC_{90}s of ticarcillin plus clavulanic acid were achieved only for *Proteus* spp., *Providencia* spp., and *Morganella* spp.

TABLE 2. Comparative activity of tazobactam, clavulanic acid, or sulbactam when combined with piperacillin versus 77 piperacillin-susceptible and moderately susceptible[a] enteric bacteria; comparison with ticarcillin plus clavulanic acid

Agent[b]	MIC (μg/ml)[c]		
	Range	50%	90%
PIP	0.12–64	4	64
PIP +			
TZB (2 μg/ml)	0.03–64	1	16
CVA (2 μg/ml)	0.03–128	2	64
SBT (2 μg/ml)	0.03–64	2	32
PIP +			
TZB (4 μg/ml)	0.03–128	1	16
CVA (4 μg/ml)	0.03–128	2	64
SBT (4 μg/ml)	0.03–64	1	16
TIC	0.25–>128	16	>128
TIC +			
CVA (2 μg/ml)	0.5–128	2	64
TZB	32–>128	>128	>128
CVA	16–128	64	128
SBT	16–>128	64	128

[a] MIC of ≤64 μg/ml.
[b] For abbreviations, see Table 1, footnote *a*.
[c] In combinations, MICs are in terms of piperacillin or ticarcillin.

The piperacillin-β-lactamase inhibitor combinations also enhanced the activity of piperacillin by 4- to 16-fold against piperacillin-susceptible (≤16 μg/ml) or moderately susceptible (32 to 64 μg/ml) enteric bacteria (Table 2). Clavulanic acid also enhanced the activity of ticarcillin against these isolates. Overall, tazobactam was the most effective inhibitor. No antagonism (an increase of ≥4 in the MICs of piperacillin) was observed with tazobactam or sulbactam. Clavulanic acid, however, effected antagonism with piperacillin versus one strain each of *Serratia* and *Morganella* and three strains of *Providencia*.

All three β-lactamase inhibitors enhanced the activity of piperacillin against oxacillin-susceptible staphylococci and β-lactamase-positive *Bacteroides fragilis* group isolates (Table 3). These strains varied in susceptibility to piperacillin or

TABLE 3. Effect of β-lactamase inhibitors on piperacillin susceptibility of β-lactamase-producing *B. fragilis* group and oxacillin-susceptible staphylococci; comparison with ticarcillin plus clavulanic acid

Organism (no. of strains tested)	Agent[a]	MIC (μg/ml)[b]		
		Range	50%	90%
B. fragilis group (28)	PIP	4–>128	128	>128
	PIP +			
	TZB 2	0.25–16	1	8
	TZB 4	0.25–8	0.5	4
	CVA 2	0.25–32	1	8
	CVA 4	0.25–16	0.5	4
	SBT 2	1–16	2	8
	SBT 4	0.5–8	1	8
	TIC	16–>128	>128	
	TIC +			
	CVA 2	0.25–32	0.5	4
	TZB	8–32	8	32
	CVA	8–32	16	16
	SBT	8–32	16	32
S. aureus (12)	PIP	4–>128	16	64
	PIP +			
	TZB 2	0.5–8	1	2
	TZB 4	0.5–2	1	2
	CVA 2	0.5–2	1	2
	CVA 4	0.5–1	1	1
	SBT 2	1–8	2	4
	SBT 4	0.5–2	2	2
	TIC	4–8	8	8
	TIC +			
	CVA 2	2–4	2	4
	TZB	32–>128	64	64
	CVA	16–64	16	64
	SBT	128–>128	128	>128
Coagulase-negative staphylococci (12)	PIP	1–>128	4	32
	PIP +			
	TZB 2	≤0.25–2	0.5	2
	TZB 4	≤0.25–1	0.5	1
	CVA 2	≤0.25–1	0.5	1
	CVA 4	≤0.25–1	0.5	1
	SBT 2	≤0.25–2	1	2
	SBT 4	≤0.25–2	0.5	2
	TIC	2–16	4	8
	TIC +			
	CVA 2	≤0.25–4	2	4
	TZB	16–>128	32	128
	CVA	4–32	8	32
	SBT	128–>128	>128	

[a] Antibiotics were tested with a fixed concentration of 2 or 4 μg of inhibitor per ml. For abbreviations, see Table 1, footnote *a*.
[b] In combinations, MICs are in terms of piperacillin or ticarcillin.

TABLE 4. Effect of 2 μg of tazobactam, clavulanic acid, or sulbactam per ml on activity of piperacillin versus specific β-lactamase-producing strains, compared with ticarcillin plus clavulanic acid

Organism, enzyme	MIC (μg/ml)[a]					
	PIP	PIP + TZB	PIP + CVA	PIP + SBT	TIC	TIC + CVA
E. coli, OXA-1	64	16	8	16		256
E. coli, OXA-2	16	1	2	1	256	4
E. coli, OXA-3	32	2	2	2	512	32
E. coli, OXA-4	32	16	16	16	512	32
P. aeruginosa, OXA-6	128	16	4	128	256	128
E. coli, OXA-7	64	32	32	32	>512	32
P. aeruginosa, PSE-1	256	64	8	64	>512	128
P. aeruginosa, PSE-2	32	32	32	32	256	128
P. aeruginosa, PSE-3	64	4	4	4	>512	16
P. aeruginosa, PSE-4	128	16	8	64	>512	128
P. aeruginosa, CARB-4	512	128	64	128	>512	128
P. aeruginosa, LCR-1	64	32	32	32	>512	>512
E. coli, TEM-1	>512	2	4	>512	>512	>512
E. coli, TEM-2	>512	4	2	>512	>512	512
E. coli, SHV-1	256	2	2	128	>128	64
K. pneumoniae, CTX-1	>512	8	8	16	>512	64
E. coli, OHIO-1	64	16	1	32		64
E. coli, HMS-1	>512	8	2	>512	>512	512
E. coli, ROB-1	512	1	1	1		128
E. coli, TLE-1	>512	2	1	16	>512	16

[a] For abbreviations, see Table 1, footnote *a*. In combinations, MICs are in terms of piperacillin or ticarcillin.

ticarcillin and were, in general, more susceptible than the gram-negative enteric bacteria to the inhibitors alone. Overall, the inhibitors effected a ≥16-fold reduction in the MICs of piperacillin for oxacillin-susceptible staphylococci. The MICs of piperacillin in combination with tazobactam or clavulanic acid were lower than those of ticarcillin combined with clavulanic acid. These staphylococcal strains were generally more susceptible to ticarcillin than to piperacillin alone, and the addition of clavulanic acid to ticarcillin had little effect. None of the inhibitor-antibiotic combinations was effective against the oxacillin-resistant *Staphylococcus* spp. that were tested. The three inhibitors were equally effective in reducing the MICs of piperacillin versus the *B. fragilis* group of bacteria. The MIC_{90}s of piperacillin were reduced greater than 16-fold by combination with the inhibitors. Ticarcillin plus clavulanic acid was similarly effective.

Tazobactam and clavulanic acid at 2 μg/ml reduced the MICs of piperacillin against the following strains producing characterized plasmid-mediated β-lactamases: *E. coli* with TEM-1, TEM-2, OXA-1, OXA-2, OXA-3, SHV-1, OHIO-1, HMS-1, ROB-1, and TLE-1; *K. pneumoniae* with CTX-1 (TEM-3); and *Pseudomonas aeruginosa* with PSE-1, PSE-3, PSE-4, and OXA-6 (Table 4). Sulbactam at 2 μg/ml did not reduce the MICs of piperacillin against the strains producing TEM-1, TEM-2, SHV-1, OHIO-1, HMS-1, and PSE-4. None of the inhibitors was effective when combined with piperacillin against strains with PSE-2, OXA-4, OXA-7, and LCR-1 β-lactamase; however, clavulanic acid enhanced the activity of ticarcillin against OXA-4 and OXA-7 enzymes. The strains with plasmid-mediated enzymes were innately more resistant to ticarcillin than to piperacillin, and although clavulanic acid lowered the MICs of ticarcillin, the reduction was not to the levels achieved with piperacillin combined with the inhibitors.

In vivo. Tazobactam was highly effective in reducing the piperacillin doses required to protect mice from five infections produced with β-lactamase-producing bacteria. The reduction in the 50% effective doses of piperacillin by clavulanic acid was similar or twofold better than those effected by tazobactam for the *E. coli*, *K. pneumoniae*, *P.*

TABLE 5. Effects of β-lactamase inhibitors on the therapeutic efficacy of piperacillin in mice infected with piperacillin-resistant bacteria

Treatment	ED_{50}[a] mg/kg, (95% confidence limits)				
	E. coli LSU-80-8	*K. pneumoniae* K-81-9	*P. mirabilis* CHUL-87-26	*M. morganii* VGH-84-11	*S. aureus* ROSE
Single agents[b]					
PIP	220 (150–310)	150 (110–200)	100 (54–200)	170 (110–230)	>512
TZB	220 (170–300)	310 (240–400)	>512	370[c]	110 (78–150)
CVA	49 (40–60)	170 (140–210)	440[c]	110 (93–140)	30 (24–38)
SBT	70 (60–90)	85 (68–110)	400[c]	200 (140–270)	190 (150–250)
PIP + inhibitor					
TZB (4:1)	9.8 (6.7–14)	15 (11–20)	15 (9–25)	12 (8.9–17)	28 (21–34)
CVA (4:1)	10 (8.4–13)	8.4 (6.8–10)	14 (10–20)	79 (65–97)	16 (13–20)
SBT (4:1)	44 (35–57)	34 (27–42)	24 (17–34)	15 (11–20)	34 (26–43)
TZB (8:1)	18 (13–22)	19 (14–25)	22 (14–35)	17 (10–23)	33 (23–45)
CVA (8:1)	12 (10–15)	11 (8.6–13)	14 (10–20)	95 (78–120)	25 (20–31)
SBT (8:1)	71 (56–92)	46 (37–58)	38 (26–55)	16 (11–22)	57 (45–72)

[a] ED_{50}, 50% effective dose. In combinations, ED_{50}s are in terms of piperacillin.
[b] For abbreviations, see Table 1, footnote *a*.
[c] Estimate.

mirabilis, and *Staphylococcus aureus* infections (Table 5). The combination of piperacillin and sulbactam was two- to fourfold less effective than piperacillin plus tazobactam for *E. coli* and *K. pneumoniae* infections, and similar (within twofold) against the *Proteus* spp., *Morganella* spp., and *S. aureus* infections. Tazobactam did not interfere with the therapeutic effect of piperacillin against an infection produced with a susceptible strain of *E. coli*. The 50% effective dose of piperacillin was 5.2 mg/kg when the agent was used alone and 3.8 mg/kg when it was administered with tazobactam in a ratio of 8:1. In general, the therapeutic effects of the combinations of piperacillin plus the inhibitors reflected the in vitro activities against these organisms (Table 6). The in vitro enhancement of the activity of piperacillin by tazobactam was similar to that of clavulanic acid, except for the *Morganella morganii* strain. Against this culture, clavulanic acid did not reduce the MIC of piperacillin and had little effect on the therapeutic doses of piperacillin in mice.

DISCUSSION

Our results show that tazobactam can extend the spectrum of piperacillin to include many β-lactamase-producing bacteria. It was superior to sulbactam and similar to clavulanic acid in extending the spectrum of activity of piperacillin against resistant strains. Jacobs et al., tested these three inhibitors with penicillin antibiotics, including piperacillin, and reported tazobactam to be the most effective inhibitor (5). Overall, our in vitro results with respect to spectrum of activity are in good accordance with those of other investigators who tested piperacillin and tazobactam combinations in ratios of 1:1 to 8:1 or with fixed concentrations of 2 to 10 μg of the inhibitor per ml. Tazobactam produced significant reductions in the MICs of piperacillin against enteric bacteria, staphylococci, or *Bacteroides* spp. tested previously (C. Roy, F. Soriano, G. Piedrola, E. Perea, F. Martin-Luengo, R. Martin, M. Gobernado, R. Gomez-Lus, J. A. Garcia-Rodriguez, J. Garcia Lomas, E. Rodenas, and F. Baquero, 28th ICAAC, abstr. no. 106, 1988; H. Mittermayer, L. Binder, and R. Waischinger, 28th ICAAC, abstr. no. 107, 1988; A. Georgopoulos, W. Graninger, S. Breyer, and M. Georgopoulos, 28th ICAAC, abstr. no. 110, 1988; H. Grimm and M. Helmerking, 28th ICAAC, abstr. no. 112, 1988; J. Jacobs and L. Verbist, 28th ICAAC, abstr. no. 116, 1988; N. X. Chin and H. C. Neu, 28th ICAAC, abstr. no. 119, 1988). The data of these investigators indicate that, in general, the MICs of piperacillin were reduced to susceptible levels in association with ≤5 μg of tazobactam per ml. Further studies are required to determine the optimal in vitro concentration of tazobactam to predict a successful clinical outcome. Nevertheless, the concentrations reported to provide enhancement are well within the limits of the peak level of the inhibitor in blood, 34 μg/ml, achieved when 500 mg of tazobactam was administered with 4 g of piperacillin to human subjects (W. K. Cheung, D. S. Greene, O. Kuye, K. Shin, A. P. Tonelli, A. Houston, M. Hibberd, R. D. Faulkner, and B. M. Silber, Program Abstr. 6th Mediterr. Cong. Chemother., abstr. no. 159, 1988).

In our evaluation, combinations of piperacillin and tazobactam were more effective against a broader spectrum of

TABLE 6. In vitro susceptibility of bacteria used for infections in mice

Organism	MIC (μg/ml) of single agents[a]				MIC (μg/ml) of piperacillin[b] combined with:					
	PIP	TZB	CVA	SBT	TZB		CVA		SBT	
					2	4	2	4	2	4
E. coli LSU-80-8	>256	256	16	32	1	1	1	0.5	128	64
K. pneumoniae K-81-9	256	256	32	64	4	2	2	2	64	32
P. mirabilis CHUL-87-26	>256	>256	32	64	0.25	0.12	0.12	0.12	0.25	0.12
M. morganii VGH-84-11	256	256	64	128	1	0.5	128	128	16	2
S. aureus ROSE	>256	32	16	128	1	0.5	0.5	0.5	1	1

[a] For abbreviations, see Table 1, footnote *a*.
[b] Piperacillin tested with fixed concentration of 2 or 4 μg of inhibitor per ml.

enteric bacteria than ticarcillin combined with clavulanic acid. Knapp et al. (C. C. Knapp, J. Sierra-Madero, and J. A. Washington, 28th ICAAC, abstr. no. 114, 1988) reported similar results for enteric bacteria by using a ratio of 8:1 of piperacillin to tazobactam. Medeiros et al. (A. A. Medeiros, J. Martinez-Beltran, E. F. Papa, and C. O'Gara, 28th ICAAC, abst. no. 491, 1988) observed that piperacillin combined with tazobactam or clavulanic was more potent than ticarcillin combined with clavulanic acid against β-lactamase-producing *E. coli* isolates. These investigators concluded that the greater inherent susceptibility of the strains to piperacillin compared with ticarcillin could account for the difference in potency. Tazobactam and clavulanic acid expanded the spectrum of activity of piperacillin to include strains that produce various plasmid-mediated β-lactamases, particularly the widely distributed TEM-1, TEM-2, and the recently described CTX-1 (TEM-3) enzymes. Our results agree with those of other investigators (3, 6). In general, clavulanic acid was more active with piperacillin than tazobactam or sulbactam. Gutman et al. suggested that the superior activity of clavulanic acid may be related to its high intrinsic activity or better penetration (3). In contrast to the report of Jacobs et al., we found that the combination of ticarcillin and clavulanic acid showed poor activity against the strains with defined β-lactamases (5). Our divergent results may be due to the difference in concentrations of clavulanic acid tested (2 versus 8 μg/ml) or the level of β-lactamase produced by the strains. King et al. tested a related pair of resistant *Klebsiella* strains and found that 2 to 8 μg of clavulanic acid per ml reduced the MIC of ticarcillin for the parent strain to the susceptible range, but not for the variant, which produced larger amounts of enzyme (A. King, W. R. Gransden, and I. Phillips, Proc. 13th Int. Congr. Chemother., p. 56–59, 1983). These investigators also found that an *E. coli* strain that produced high levels of TEM-2 was not rendered susceptible to ticarcillin plus clavulanic acid.

In addition to the species tested in this study, tazobactam is reported to be effective in lowering MICs of piperacillin for β-lactamase-positive strains of *Haemophilus influenzae* and *Branhamella catarrhalis* (E. J. Perea, M. C. Garcia-Iglesias, and M. J. Clavijo, 28th ICAAC, abstr. no. 113, 1988; N. A. Kuck, P. J. Petersen, W. J. Weiss, N. V. Jacobus, R. T. Testa, and F. P. Tally, Program Abstr. 6th Mediterr. Cong. Chemother., abstr. no. 155, 1988). Also, in comparison to clavulanic acid, tazobactam is a poor inducer of chromosomal β-lactamases (9).

In addition to the in vitro effects, our tests show that tazobactam in combination with piperacillin is therapeutically effective in mice infected with piperacillin-resistant bacteria. Thus a piperacillin-tazobactam combination could be a valuable approach to the chemotherapy of infections caused by β-lactamase-containing bacteria.

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