ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 1988, p. 84–91
0066-4804/88/010084-08$02.00/0

Vol. 32, No. 1

Tigemonam, an Oral Monobactam

NAI-XUN CHIN[1] AND HAROLD C. NEU[1,2]*

Departments of Medicine[1] and Pharmacology,[2] College of Physicians & Surgeons, Columbia University, 630 West 168 Street, New York, New York 10032

Received 29 June 1987/Accepted 23 October 1987

Tigemonam is an orally administered monobactam. At ≤1 μg/ml it inhibited the majority of strains of *Escherichia coli*, *Klebsiella* spp., *Enterobacter aerogenes*, *Citrobacter diversus*, *Proteus* spp., *Providencia* spp., *Aeromonas hydrophila*, *Salmonella* spp., *Shigella* spp., *Serratia marcescens*, and *Yersinia enterocolitica*. At ≤0.25 μg/ml it inhibited *Haemophilus* spp., *Neisseria* spp., and *Branhamella catarrhalis*. It did not inhibit *Pseudomonas* spp. or *Acinetobacter* spp. Tigemonam was more active than cephalexin and amoxicillin-clavulanate and inhibited many members of the family *Enterobacteriaceae* resistant to trimethoprim-sulfamethoxazole and gentamicin. Some *Enterobacter cloacae* and *Citrobacter freundii* strains resistant to aminothiazole iminomethoxy cephalosporins and aztreonam were resistant to tigemonam. The MIC for 90% of hemolytic streptococci of groups A, B, and C and for *Streptococcus pneumoniae* was 16 μg/ml, but the MIC for 90% of enterococci, *Listeria* spp., *Bacteroides* spp., and viridans group streptococci was >64 μg/ml. Tigemonam was not hydrolyzed by the common plasmid β-lactamases such as TEM-1 and SHV-1 or by the chromosomal β-lactamases of *Enterobacter*, *Morganella*, *Pseudomonas*, and *Bacteroides* spp. Tigemonam inhibited β-lactamases of *E. cloacae* and *Pseudomonas aeruginosa* but did not induce β-lactamases. The growth medium had a minimal effect on the in vitro activity of tigemonam, and there was a close agreement between the MICs and MBCs.

The discovery in 1978 of the monobactam antibiotics led to the discovery of a new family of beta-lactam agents. Aztreonam, the first of these agents, has undergone extensive in vitro, pharmacological, and clinical studies and recently has been approved for clinical use in the United States (1, 4, 7, 12–15). Other monobactam agents have been synthesized, and carumonam is also currently undergoing clinical investigation in Japan and the United States. Although several monobactams have been shown to be absorbed after oral ingestion in rodents, tigemonam is the first monobactam which is absorbed well after oral administration in both laboratory animals and humans (3, 16). This investigation was performed to compare the in vitro activity of tigemonam with those of other oral agents and selected agents for which it could be considered follow-up oral therapy.

MATERIALS AND METHODS

Drugs. Tigemonam and aztreonam were provided by E. R. Squibb & Sons, Princeton, N.J.; cephalexin was provided by Eli Lilly & Co., Indianapolis, Ind.; amoxicillin-clavulanate was provided by Beecham Laboratories, Bristol, Tenn.; trimethoprim-sulfamethoxazole was provided by Roche Diagnostics, Div. Hoffmann-La Roche Inc., Nutley, N.J.; ciprofloxacin was provided by Miles Laboratories, Inc., West Haven, Conn.; and gentamicin was provided by Schering Corp., Bloomfield, N.J.

Bacteria. Bacterial isolates were from patients recently hospitalized or seen in outpatient departments of The Presbyterian Hospital in New York City. Selected isolates resistant to beta-lactams were sent to our laboratory from the other eight hospitals in the Columbia University system. The presence of β-lactamases in the isolates was determined by the nitrocefin spot assay (5).

Susceptibility testing. Antimicrobial activity was measured by an agar dilution method with Mueller-Hinton agar and a spot-replicating device that applied 10^5 CFU. Broth dilutions were performed with 5×10^5 CFU in 1-ml tubes. Incubation of cultures was done at 35°C for 18 to 20 h. The MIC was defined as the lowest concentration of antibiotic which inhibited the development of visible growth on agar or in broth. The MBC was determined by plating 0.01 ml from clear tubes to antibiotic-free agar and examining the plates after 24 h of incubation at 35°C. Tests were performed in duplicate, and CFU were determined with rejection values as described by Pearson et al. (9). The MBC was defined as the lowest concentration of antibiotic that killed 99.9% of the organisms. The susceptibility of *Neisseria*, *Branhamella*, and *Haemophilus* species was determined with chocolatized agar to which IsoVitaleX had been added, and incubation was done in the presence of 5% CO_2 for 18 h at 35°C.

β-Lactamase assays and inhibition studies. β-Lactamases used for analysis of the stability of tigemonam were partially purified enzymes, as previously described (5). Stability against β-lactamases was determined by using a computerized, heat-controlled spectrophotometer (Response; Gilford Instrument Laboratories, Inc., Oberlin, Ohio). The change in the absorbance of each substrate at its absorption maximum at a 0.1 mM concentration was monitored. Inhibition assays were done with equimolar concentrations of cephaloridine and tigemonam. Enzymes were preincubated with tigemonam for 10 min at 35°C before cephaloridine was added to the mixture.

Induction of β-lactamases. Bacterial strains were grown overnight in Mueller-Hinton broth and diluted 20-fold into fresh broth. After 2 h of incubation in a rotary shaker at 35°C, tigemonam was added at concentrations of 0.1, 0.5, and 1 μg/ml. Incubation was continued for 2 h, and bacteria were harvested by centrifugation, washed in 0.05 M potassium phosphate buffer (pH 7), and disrupted by sonication. Cell debris was removed by centrifugation. The supernatant material was dialyzed at 4°C for 24 h in the aforementioned buffer and stored at −20°C until assayed. β-Lactamase

* Corresponding author.

TABLE 1. Comparative activity of tigemonam against gram-negative bacteria

Organism (no. tested)	Antibiotic	MIC (μg/ml)[a]		
		Range	50%	90%
Escherichia coli (30)[b]	Tigemonam	0.03–1	0.25	0.5
	Aztreonam	0.015–0.25	0.06	0.25
	Cephalexin	4–>64	8	32
	Amoxicillin-clavulanate[c]	2–32	8	16
	TMP-SMX	0.12–>64	0.12	>64
	Gentamicin	0.25–2	0.5	1
	Ciprofloxacin	≤0.008–0.015	0.008	0.015
Klebsiella pneumoniae (30)[b]	Tigemonam	0.03–2	0.25	1
	Aztreonam	0.015–1	0.25	1
	Cephalexin	4–>64	8	32
	Amoxicillin-clavulanate[c]	0.25–64	16	32
	TMP-SMX	0.1–>64	4	>64
	Gentamicin	0.25–>16	16	>16
	Ciprofloxacin	≤0.008–0.015	≤0.008	0.015
Klebsiella oxytoca (20)[b]	Tigemonam	0.06–2	0.25	0.5
	Aztreonam	0.03–8	0.25	4
	Cephalexin	4–>64	8	>64
	Amoxicillin-clavulanate[c]	1–>32	8	>32
	TMP-SMX	0.06–16	0.25	8
	Gentamicin	0.5–>32	2	32
	Ciprofloxacin	0.015–0.5	0.06	0.12
Enterobacter aerogenes (30)	Tigemonam	0.12–4	0.5	1
	Aztreonam	≤0.06–16	0.12	4
	Cephalexin	>32	>32	>32
	Amoxicillin-clavulanate[c]	2–64	64	64
	TMP-SMX	0.12–1	0.25	0.5
	Gentamicin	0.5–>16	2	8
	Ciprofloxacin	0.015–0.25	0.015	0.03
Enterobacter cloacae (30)[b]	Tigemonam	0.12–16	1	8
	Aztreonam	≤0.06–32	0.25	32
	Cephalexin	>16	>16	>16
	Amoxicillin-clavulanate[c]	16–64	64	64
	TMP-SMX	0.12–>16	0.25	>16
	Gentamicin	0.5–128	2	64
	Ciprofloxacin	≤0.008–0.12	0.015	0.03
Hafnia alvei (17)[b]	Tigemonam	0.12–0.5	0.25	0.5
	Aztreonam	0.12–1	0.25	0.5
	Cephalexin	>32	>32	>32
	Amoxicillin-clavulanate[c]	1–64	64	64
	TMP-SMX	0.21–1	0.25	0.25
	Gentamicin	0.25–1	0.5	1
	Ciprofoxacin	0.015–0.12	0.03	0.12
Citrobacter freundii (20)[b]	Tigemonam	0.12–8	0.25	2
	Aztreonam	0.06–16	0.25	16
	Cephalexin	>32	>32	>32
	Amoxicillin-clavulanate[c]	16–64	64	64
	TMP-SMX	0.06–>16	0.25	>16
	Gentamicin	0.25–>16	1	2
	Ciprofloxacin	≤0.008–1	0.06	0.06
Citrobacter diversus (20)	Tigemonam	0.03–1	0.25	0.25
	Aztreonam	≤0.0015–0.06	0.03	0.06
	Cephalexin	4–8	8	9
	Amoxicillin-clavulanate[c]	1–16	2	4
	TMP-SMX	0.06–0.25	0.12	0.25
	Gentamicin	0.25–2	0.5	0.5
	Ciprofloxacin	≤0.008	≤0.008	≤0.008
Proteus mirabilis (30)	Tigemonam	≤0.015–0.25	≤0.015	0.03
	Aztreonam	≤0.015–0.06	≤0.015	≤0.015
	Cephalexin	>32	>32	>32
	Amoxicillin-clavulanate[c]	0.5–8	1	8

Continued on following page

TABLE 1—*Continued*

Organism (no. tested)	Antibiotic	MIC (μg/ml)[a]		
		Range	50%	90%
	TMP-SMX	≤0.06–8	≤0.06	0.5
	Gentamicin	1–8	2	4
	Ciprofloxacin	≤0.008–0.5	0.015	0.12
Morganella morganii (30)[b]	Tigemonam	≤0.015–1	0.03	0.12
	Aztreonam	≤0.015	≤0.008	0.008
	Cephalexin	>32	>32	>32
	TMP-SMX	≤0.06–8	0.06	0.06
	Gentamicin	0.25–16	1	4
	Ciprofloxacin	≤0.008–0.5	≤0.008	0.06
Proteus vulgaris (30)[b]	Tigemonam	≤0.015–0.25	0.03	0.25
	Aztreonam	≤0.015–0.25	0.12	0.25
	Cephalexin	>32	>32	>32
	Amoxicillin-clavulanate[c]	2–>16	8	>16
	TMP-SMX	0.06–>16	0.25	2
	Gentamicin	0.12–>16	0.5	8
	Ciprofloxacin	≤0.008–4	0.015	0.06
Proteus rettgeri (20)[b]	Tigemonam	≤0.015–>0.25	0.06	>0.12
	Aztreonam	0.03–>0.12	0.03	>0.12
	Cephalexin	>32	>32	>32
	Amoxicillin-clavulanate[c]	2–16	8	>16
	TMP-SMX	0.06–>16	0.25	8
	Gentamicin	0.12–>16	4	>16
	Ciprofloxacin	≤0.008–2	0.5	2
Providencia stuartii (20)[b]	Tigemonam	≤0.015–1	≤0.015	0.06
	Aztreonam	≤0.015–1	≤0.015	0.03
	Cephalexin	>32	>32	>32
	Amoxicillin-clavulanate[c]	8–>32	>32	>32
	TMP-SMX	0.12–>16	0.25	16
	Gentamicin	4–>16	16	>16
	Ciprofloxacin	0.06–2	0.25	1
Serratia marcescens (30)[b]	Tigemonam	0.12–0.5	0.25	0.5
	Aztreonam	0.12–4	0.25	0.5
	Cephalexin	>32	>32	>32
	Amoxicillin-clavulanate[c]	>16	>16	>16
	TMP-SMX	0.25–>16	0.5	4
	Gentamicin	0.5–16	2	8
	Ciprofloxacin	0.06–1	0.12	0.5
Pseudomonas aeruginosa (20)[b]	Tigemonam	64–>64	>64	>64
	Aztreonam	4–32	8	16
	Gentamicin	0.25–>16	4	>16
	Ciprofloxacin	0.015–2	0.12	0.25
Pseudomonas cepacia (10)[b]	Tigemonam	0.12–128	1	2
	Aztreonam	0.12–64	8	32
	TMP-SMX	0.5–>16	0.5	0.5
	Gentamicin	2–>32	>32	>32
	Ciprofloxacin	0.12–8	2	4
Pseudomonas spp. (30)[b,d]	Tigemonam	16–>128	>128	>128
	Aztreonam	4–>128	128	>128
	TMP-SMX	0.5–>16	0.5	>16
	Gentamicin	>32	>32	>32
	Ciprofloxacin	0.25–4	0.12	4
Acinetobacter calcoaceticus (30)[b]	Tigemonam	128–>128	>128	>128
	Aztreonam	16–>128	128	>128
	Cephalexin	>32	>32	>32
	Amoxicillin-clavulanate[c]	4–64	16	32
	TMP-SMX	0.25–8	0.5	1
	Gentamicin	0.25–>64	1	2
	Ciprofloxacin	0.03–4	0.5	1

Continued on following page

TABLE 1—*Continued*

Organism (no. tested)	Antibiotic	MIC (μg/ml)[a]		
		Range	50%	90%
Salmonella spp. (35)[b]	Tigemonam	0.06–1	0.25	1
	Aztreonam	0.015–0.5	0.12	0.5
	Cephalexin	4–>32	16	16
	Amoxicillin-clavulanate[c]	0.5–>16	4	>16
	TMP-SMX	0.06–16	0.12	0.5
	Gentamicin	0.25–4	0.5	4
	Ciprofloxacin	≤0.008–0.12	≤0.008	0.03
Shigella spp. (35)[b]	Tigemonam	0.25–0.5	0.25	0.5
	Aztreonam	0.06–0.5	0.12	0.25
	Cephalexin	8–>32	8	>32
	Amoxicillin-clavulanate[c]	4–>16	4	>16
	TMP-SMX	0.06–16	0.12	0.5
	Gentamicin	0.25–2	1	2
	Ciprofloxacin	≤0.008–0.03	≤0.008	0.03
Aeromonas hydrophila (10)[b]	Tigemonam	0.03–0.12	0.06	0.12
	Aztreonam	≤0.015–0.25	0.03	0.03
	Cephalexin	>32	>32	>32
	Amoxicillin-clavulanate[c]	4–>16	16	>16
	TMP-SMX	0.25–0.5	0.5	0.5
	Gentamicin	1–4	2	4
	Ciprofloxacin	≤0.008–0.06	≤0.008	0.06
Yersinia enterocolitica (20)[b]	Tigemonam	0.03–0.5	0.12	0.25
	Aztreonam	0.03–2	0.25	1
	Cephalexin	>32	>32	>32
	Amoxicillin-clavulanate[c]	0.5–>16	4	16
	TMP-SMX	0.06–0.5	0.12	0.1
	Gentamicin	0.5–4	1	2
	Ciprofloxacin	≤0.008–0.06	0.03	0.03
Haemophilus influenzae (21)[e]	Tigemonam	0.06–0.25	0.12	0.12
	Aztreonam	0.03–0.25	0.06	0.12
	Cephalexin	1–>32	4	>32
	Amoxicillin-clavulanate[c]	0.25–1	0.25	0.5
	TMP-SMX	0.25–0.5	0.25	0.5
	Gentamicin	1–4	4	4
	Ciprofloxacin	<0.008–0.015	0.015	0.015
Haemophilus parainfluenzae (5)	Tigemonam	0.06–0.25		
Neisseria gonorrhoeae (20)[e]	Tigemonam	0.015–0.12	0.03	0.06
	Aztreonam	0.015–0.2	0.03	0.06
	Cephalexin	1–16	8	16
	Ciprofloxacin	<0.015	≤0.015	≤0.015
Branhamella catarrhalis (20)[e]	Tigemonam	≤0.06–0.12	0.12	0.12
	Aztreonam	0.12–0.25	0.12	0.25
	Cephalexin	4–>32	4	>32
	Amoxicillin-clavulanate[c]	≤0.12–1	≤0.12	1
	Gentamicin	≤0.12–>16	1	>16
	Ciprofloxacin	≤0.12	≤0.12	≤0.12

[a] 50% and 90%, MIC for 50 and 90% of strains, respectively.
[b] All of the isolates were ampicillin resistant.
[c] Amoxicillin and clavulanate were used at a 2:1 concentration.
[d] *Pseudomonas maltophilia*, *Pseudomonas acidovorans*, and *Pseudomonas fluorescens*.
[e] Half of the isolates were β-lactamase positive.

activity was defined as the amount of enzyme which hydrolyzed 1 μM nitrocefin per min at 30°C. Antagonism of the activity of piperacillin and cefamandole was determined by the agar disk placement method of Waterworth and Emmerson (17).

Permeability studies. Permeability studies were performed with the mutant *Escherichia coli* strains of Richmond et al. (10) by determining the median MIC in six tests for each strain.

Development of resistance. The frequency of spontaneous single-step resistance to concentrations four and eight times the MIC of tigemonam was determined by plating 10^{10} bacteria, obtained by centrifugation, onto Mueller-Hinton agar containing the antibiotic at concentrations four and

eight times the MIC. CFU were determined after 48 h of incubation at 35°C.

RESULTS

Susceptibility testing. The antibacterial activity of tigemonam against aerobic gram-negative bacteria is shown in Table 1. Tigemonam at ≤1 μg/ml inhibited the majority of strains of *E. coli*, *Klebsiella* spp., *Enterobacter aerogenes*, *Hafnia alvei*, *Citrobacter diversus*, *Proteus mirabilis*, *Morganella morganii*, *Proteus vulgaris*, *Proteus rettgeri*, *Providencia* spp., *Serratia marcescens*, *Salmonella* spp. (including *Salmonella typhi*), *Shigella* spp., *Aeromonas hydrophila*, *Yersinia enterocolitica*, *Haemophilus influenzae*, *Haemophilus parainfluenzae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis* (six isolates not shown), and *Branhamella catarrhalis*.

Tigemonam was more active than aztreonam against *Klebsiella oxytoca*, *Enterobacter* spp., and *Citrobacter freundii*, but with other species aztreonam usually was more active by one dilution. Tigemonam did not inhibit *Pseudomonas aeruginosa* but was more active than aztreonam against *Pseudomonas cepacia* and, like aztreonam, tigemonam did not inhibit *Acinetobacter* spp.

Tigemonam was considerably more active than cephalexin and amoxicillin-clavulanate in inhibiting members of the family *Enterobacteriaceae* resistant to both agents. Tigemonam also inhibited *E. coli* and *Klebsiella*, *Proteus*, *Providencia*, and *Serratia* spp. resistant to trimethoprim-sulfamethoxazole (TMP-SMX). It also inhibited *Salmonella* and *Shigella* spp. resistant to amoxicillin, TMP-SMX, and chloramphenicol (data not shown). *Klebsiella* spp., most *Enterobacter* spp., and *S. marcescens* isolates resistant to gentamicin were inhibited by ≤2 μg of tigemonam per ml.

Similar to the MICs of virtually all other new beta-lactams, tigemonam MICs for some *Enterobacter cloacae* and *C. freundii* isolates were 8 to 16 μg/ml. For these same isolates, cefotaxime, ceftazidime, and cefoperazone MICs were ≥32 μg/ml, but the isolates were inhibited by 4 μg of imipenem per ml (data not shown). Overall, ciprofloxacin, the quinolone used for comparison, was more active than any of the other agents tested by 32- to 128-fold.

The activity of tigemonam against gram-positive and anaerobic bacteria is shown in Table 2. It had virtually no activity against staphylococci, but hemolytic streptococci were inhibited at concentrations of 1 to 16 μg/ml, as compared with 16 to >128 μg of aztreonam per ml. The same was true for *Streptococcus pneumoniae* and *Clostridium perfringens*, but *Streptococcus faecalis*, viridans group streptococci, *Listeria monocytogenes*, and *Bacteroides fragilis* were completely resistant to tigemonam, as they were to aztreonam.

Effect of growth conditions upon MICs and MBCs. The in vitro activity of tigemonam was similar in Mueller-Hinton, brain heart infusion, and tryptic digest agar media for the members of the family *Enterobacteriaceae* tested (five isolates each of *E. coli*, *Klebsiella pneumoniae*, *S. marcescens*, *Providencia stuartii*, and *Enterobacter* spp.). Tigemonam MBCs were two- to fourfold higher than the MICs, except for *P. mirabilis* (Table 3), and in general the MBCs were <2 μg/ml. All of the isolates tested were ampicillin and cephalexin resistant. The effect of variation of the inoculum size is shown in Table 4. The MICs were slightly lower at 10^3 CFU than at 10^5 CFU, and at 10^7 CFU the MICs increased from ≤0.12 to ≥4 μg/ml for *K. pneumoniae* and *Morganella morganii*.

The activity of tigemonam in urine and human serum

TABLE 2. Activity of tigemonam against gram-positive and anaerobic bacteria

Organism (no. tested)	Antibiotic	MIC (μg/ml)[a]		
		Range	50%	90%
Staphylococcus aureus (20)[b]	Tigemonam	>128	>128	>128
	Aztreonam	>128	>128	>128
Staphylococcus epidermidis (20)[b]	Tigemonam	>128	>128	>128
	Aztreonam	>128	>128	>128
Group A, B, C, G, and F streptococci (20)	Tigemonam	1–6	8	8
	Aztreonam	>32	>32	>32
Streptococcus pneumoniae (10)	Tigemonam	8–32	8	16
	Aztreonam	>32	>32	>32
Streptococcus faecalis (10)	Tigemonam	>128	>128	>128
	Aztreonam	>128	>128	>128
Viridans group streptococci (20)	Tigemonam	>128	>128	>128
	Aztreonam	>128	>128	>128
Listeria monocytogenes (10)	Tigemonam	>128	>128	>128
	Aztreonam	>128	>128	>128
Clostridium perfringens (10)	Tigemonam	8–>32	16	16
	Aztreonam	>32	>32	>32
Bacteroides fragilis (10)	Tigemonam	>128	>128	>128
	Aztreonam	>128	>128	>128

[a] See Table 1, footnote *a*.
[b] Includes five methicillin-resistant isolates.

TABLE 3. Comparison of MICs and MBCs of tigemonam

Organism[a]	MIC (μg/ml)		MBC (μg/ml)	
	Range	Geometric mean	Range	Geometric mean
Escherichia coli	0.12–1	0.3	0.25–2	0.6
Klebsiella pneumoniae	0.06–2	0.5	0.25–8	1.7
Citrobacter freundii	0.06–1	0.4	0.5–4	1.7
Enterobacter cloacae	0.06–4	0.3	0.06–4	0.6
Morganella morganii	0.03–0.25	0.1	0.25–2	0.7
Proteus mirabilis	0.03–0.12	0.05	0.12–8	1.5
Serratia marcescens	0.25–1	0.4	0.5–8	1.5

[a] Five isolates of each species were tested.

TABLE 4. Effect of inoculum size on MICs of tigemonam

Organism[a]	Geometric mean MIC (μg/ml) at inoculum size of:		
	10^3 CFU	10^5 CFU	10^7 CFU
Escherichia coli	0.1	0.22	0.5
Klebsiella pneumoniae	0.1	0.22	4.5
Enterobacter cloacae	0.18	0.35	2.5
Morganella morganii	0.03	0.04	4
Serratia marcescens	0.18	0.4	1.8

[a] Six isolates of each species were tested.

TABLE 5. Effect of growth medium on MICs and MBCs of tigemonam[a]

Organism	Mueller-Hinton broth				Urine (pH 5.5)		MBC in human serum[b] (pH 7.4)
	pH 7.4		pH 5.5				
	MIC	MBC	MIC	MBC	MIC	MBC	
Escherichia coli 5441	0.05	0.5	1	1	1	1	1
Escherichia coli 23	0.25	0.5	0.25	0.5	0.5	2	1
Klebsiella penumoniae 107	0.25	0.5	1	1	1	0.5	2
Klebsiella pneumoniae 109	0.5	1	2	2	0.5	1	4
Serratia marcescens 204	0.12	0.5	1	8	0.25	2	4
Serratia marcescens 187	1	1	4	4	0.25	1	4

[a] MICs and MBCs are given in micrograms per milliliter.
[b] Heat-inactivated pooled normal human serum.

TABLE 6. Frequency of spontaneous resistance to tigemonam

Organism	Frequency of resistance to:	
	Four times the MIC	Eight times the MIC
Escherichia coli 5441	$<1.2 \times 10^{-10}$	$<1.2 \times 10^{-10}$
Escherichia coli 5489	$<1.6 \times 10^{-10}$	$<1.6 \times 10^{-10}$
Klebsiella pneumoniae 104	$<1.7 \times 10^{-10}$	$<1.7 \times 10^{-10}$
Klebsiella pneumoniae 107	$<5.0 \times 10^{-9}$	$<5.0 \times 10^{-9}$
Serratia marcescens 203	$<1.2 \times 10^{-10}$	$<1.2 \times 10^{-10}$
Serratia marcescens 204	$<1.4 \times 10^{-10}$	$<1.4 \times 10^{-10}$

against representative isolates of three species is shown in Table 5. The MBC in serum was two- to fourfold higher than that in pH 7.4 Mueller-Hinton broth; the MBC in urine was equal to or twofold higher than that in pH 7.4 broth.

Activity of tigemonam against permeability mutants. The activity of tigemonam against various *E. coli* mutants with altered outer membrane proteins was determined. Tigemonam was more active by two-, four-, or eightfold against the mutants than it was against the parent strain, *E. coli* UB1005. Against these same strains the MICs of a drug such as cephaloridine showed no differences; the MICs of cefotaxime (6; data not shown) were also four- to eightfold lower for the mutants.

Frequency of spontaneous single-step resistance to tigemonam. For *E. coli*, *K. pneumoniae*, and *S. marcescens*, the frequency of spontaneous single-step resistance to concentrations four- or eightfold higher than the MIC was infrequent and was, in general, $<10^{-10}$ (Table 6).

β-Lactamase studies. The stability of tigemonam against attack by various plasmid-mediated and chromosomally mediated β-lactamases was determined. Tigemonam was not appreciably hydrolyzed by the important plasmid β-lactamases TEM-1, TEM-2, and SHV-1, (Table 7). These same enzymes under similar conditions hydrolyzed cefoperazone at relative rates of 60, 70, and 50 and cefamandole at relative rates of 55, 74, and 57, respectively. There was virtually no hydrolysis of tigemonam by a *K. oxytoca* chromosomal β-lactamase that hydrolyzed aztreonam at a relative rate of 5. There also was minimal hydrolysis of tigemonam by the important chromosomal β-lactamase of *E. cloacae* (P99), the *C. freundii* β-lactamase, or the inducible chromosomal β-lactamase of *P. aeruginosa* (Sabath-Abraham enzyme). Plasmid β-lactamases of *B. catarrhalis* and *Staphylococcus aureus* and chromosomal β-lactamases of *Bacteroides fragilis* did not hydrolyze tigemonam.

The β-lactamase-inhibiting activity of tigemonam is shown in Table 8. Tigemonam, like aminothiazole cephalosporins, was a poor inhibitor of the common plasmid β-lactamase TEM-1, but it was an effective inhibitor of chromosomal β-lactamases such as *E. cloacae* P99 and the *P. aeruginosa* Sabath-Abraham enzyme. In this sense, it was similar to aztreonam and cefotaxime and unlike clavulanate. The K_i of tigemonam for TEM-1 was 50.8 μM, as compared with a K_i of 0.86 μM for P99.

TABLE 7. β-Lactamase stability of tigemonam

Source of β-lactamase	Genetic origin	Richmond-Sykes type (trivial name)	Relative rate of hydrolysis[a] of:	
			Tigemonam	Aztreonam
Escherichia coli	Plasmid	III (TEM-1)	<0.1	<0.1
Escherichia coli	Plasmid	III (TEM-2)	<0.1	<0.1
Klebsiella pneumoniae	Plasmid	III (SHV-1)	<0.1	<0.1
Enterobacter aerogenes	Chromosomal	Ia (P99)	<0.1	<0.1
Pseudomonas aeruginosa	Chromosomal	Id (Sabath-Abraham)	<0.1	<0.1
Proteus vulgaris	Chromosomal	Id	0.6	<0.1
Klebsiella oxytoca	Chromosomal	K-1	0.4	5.0
Pseudomonas aeruginosa	Plasmid	PSE-1	0.3	<0.1
Pseudomonas aeruginosa	Plasmid	PSE-4	<0.1	<0.1
Branhamella catarrhalis	Plasmid		<0.1	<0.1
Staphylococcus aureus	Plasmid		<0.1	<0.1
Bacteroides fragilis	Chromosomal		<0.1	<0.1
Pseudomonas cepacia	Chromosomal		<0.1	<0.1

[a] Compared with the rate of hydrolysis of cephaloridine, taken to be 100. The K_m of P99 for cephaloridine was 4.9 mM. The K_m of TEM-1 for cephaloridine was 0.3 mM.

TABLE 8. Inhibition by tigemonam of hydrolysis of cephaloridine by β-lactamases

β-Lactamase and/or Richmond-Sykes type	Source of β-lactamase	% Inhibition of hydrolysis of cephaloridine by[a]:			
		Tigemonam	Aztreonam	Cefotaxime	Clavulanate
TEM-1, III	*Escherichia coli*	56	57	52	99
SHV-1, III	*Klebsiella pneumoniae*	79	ND[b]	ND	99
P99, Ia	*Enterobacter cloacae*	95	96	94	51
Ic	*Proteus vulgaris*	<0.1	ND	ND	92
Sabath-Abraham, Id	*Pseudomonas aeruginosa*	94	92	92	15

[a] The inhibitor and cephaloridine were present at equimolar concentrations.
[b] ND, Not determined.

The β-lactamase-inducing ability of tigemonam was examined for single isolates of *E. cloacae*, *S. marcescens*, and *C. freundii*. Tigemonam was not an active inducer of β-lactamases in these species (Table 9), which had previously been shown to have β-lactamases inducible by cefoxitin and other beta-lactams (6). No reduction of the zone size of piperacillin or cefamandole was found for *E. cloacae* when tested with a disk containing tigemonam.

DISCUSSION

The monobactams are a new family in the class of beta-lactam antibiotics. Although it has been possible to synthesize monocyclic beta-lactams with activity against many different bacteria, most of the compounds which have been developed inhibit aerobic gram-negative bacteria. Aztreonam has undergone extensive clinical investigation (4, 12, 13) and is currently used in the United States, Europe, and Japan to treat gram-negative infections of the respiratory and urinary tracts and gynecological and intra-abdominal infections.

No oral monobactams were available before tigemonam. As this study shows, tigemonam has in vitro activity similar to that of aztreonam, with the notable exception that it fails to inhibit *P. aeruginosa*. This result is related to the moiety on the β-acyl side chain which does not produce the antipseudomonas activity of the iminopropyl carboxyl moiety of aztreonam. The iminocarboxy substituent, however, does provide tigemonam with some activity against gram-positive aerobic hemolytic streptococci, even though this activity is much lower than that of penicillins or cephalosporins. The two methyl groups at position 4 provide tigemonam with greater stability against attack by the K-1 β-lactamase of *K. oxytoca*. We previously showed that a β-methyl compound similar to aztreonam was more active against bacteria possessing the K-1 β-lactamase (8). In this study tigemonam had activity against enteric organisms resistant to cephalexin, amoxicillin-clavulanate, and even TMP-SMX. It also inhibited selected gentamicin-resistant members of the family *Enterobacteriaceae* at concentrations of ≤2 μg/ml.

TABLE 9. Induction of β-lactamase activity by tigemonam

Organism	Ratio of β-lactamase activity of derepressed strain/that of wild-type strain at tigemonam concn (μg/ml)[a] of:		
	0.1	0.5	1
Citrobacter freundii 8375	1.3	0.4	1.6
Enterobacter cloacae 8917	1.7	0.5	1.3
Serratia marcescens 1917	1.3	1.7	1.9

[a] The MICs of tigemonam for *C. freundii* 8375, *E. cloacae* 8917, and *S. marcescens* 1917 were 0.5, 1, and 0.25 μg/ml, respectively.

Tigemonam was β-lactamase stable and was not an important inducer of β-lactamases, even though it had a high affinity for β-lactamases of chromosomal origin such as those found in *E. cloacae* and *C. freundii*. This result is similar to what has been reported for aztreonam (2, 7). We did not examine the low concentrations of tigemonam that would be present in the periplasmic space. It is probable that some destruction of tigemonam occurs, as with other β-lactamase-stable agents.

This study indicates that tigemonam is a very promising oral beta-lactam that has an extended spectrum of antibacterial activity when compared with currently available oral penicillins or cephalosporins. Our results are similar to those of Tanaka et al. (16). Aztreonam has been shown not to cause type 1 anaphylactic reactions in penicillin-allergic patients (11), and it is probable that tigemonam will be similar. Further studies of the pharmacology and clinical efficacy of this agent will determine its role in clinical infections.

LITERATURE CITED

1. **Barry, A. L., C. Thornsberry, R. N. Jones, and T. L. Gavan.** 1985. Aztreonam: antibacterial activity, β-lactamase stability, and interpretive standards and quality control guidelines for disk-diffusion susceptibility tests. Rev. Infect. Dis. 7(Suppl. 4):594–604.
2. **Bush, K., J. S. Freundenberger, and R. B. Sykes.** 1982. Interaction of aztreonam and related monobactams with β-lactamases from gram-negative bacteria. Antimicrob. Agents Chemother. 22:414–420.
3. **Clark, J. M., S. J. Olsen, D. S. Weinberg, M. D. Dalvi, R. R. Whitney, D. P. Bonner, and R. B. Sykes.** 1987. In vivo evaluation of tigemonam, a novel oral monobactam. Antimicrob. Agents Chemother. 31:226–229.
4. **Hara, K., H. Kobayashi, T. Nishiura, J. Yura, and A. Saito.** 1985. Clinical studies of aztreonam in Japan. Rev. Infect. Dis. 7(Suppl. 4):810–824.
5. **Neu, H. C.** 1986. Antibiotic inactivating enzymes and bacterial resistance, p. 757–789. *In* V. Lorian (ed.), Antibiotics in laboratory medicine, 2nd ed. The Williams & Wilkins Co., Baltimore.
6. **Neu, H. C., N. X. Chin, K. Jules, and P. Labthavikul.** 1986. The activity of BMY 28142, a new broad spectrum beta-lactamase stable cephalosporin. J. Antimicrob. Chemother. 17:441–452.
7. **Neu, H. C., and P. Labthavikul.** 1981. Antibacterial activity of a monocyclic beta-lactam, SQ 26,776. J. Antimicrob. Chemother. 8(Suppl. E):111–122.
8. **Neu, H. C., and P. Labthavikul.** 1983. In vitro activity and β-lactamase stability of a monobactam, SQ 26,917, compared with those of aztreonam and other agents. Antimicrob. Agents Chemother. 24:227–232.
9. **Pearson, R. D., R. T. Steigbigel, H. T. Davis, and S. W. Chapman.** 1980. Method for reliable determination of minimal lethal antibiotic concentrations. Antimicrob. Agents Chemother. 18:699–708.
10. **Richmond, M. H., D. C. Clark, and W. Wotton.** 1976. Indirect method for assessing the penetration of beta-lactamase-non-

susceptible penicillins and cephalosporins in *Escherichia coli* strains. Antimicrob. Agents Chemother. **10**:215–218.
11. **Saxon, A., E. A. Swabb, and N. F. Adkinson, Jr.** 1985. Investigation into the immunologic cross-reactivity of aztreonam with other beta-lactam antibiotics. Am. J. Med. **72**:19–26.
12. **Scully, B. E., and S. A. Henry.** 1985. Clinical experience with aztreonam in the treatment of gram-negative bacteremia. Rev. Infect. Dis. **7**(Suppl. 4):789–793.
13. **Scully, B. E., and H. C. Neu.** 1985. Use of aztreonam in the treatment of serious infections due to multiresistant gram-negative organisms, including *Pseudomonas aeruginosa*. Am. J. Med. **78**:251–261.
14. **Swabb, E. A.** 1985. Clinical pharmacology of aztreonam in healthy recipients and patients: a review. Rev. Infect. Dis. **7**(Suppl 4.):605–612.
15. **Sykes, R. B., D. P. Bonner, K. Bush, and N. H. Georgopapdakou.** 1982. Aztreonam (SQ,26776), a synthetic monobactam specifically active against aerobic gram-negative bacteria. Antimicrob. Agents Chemother. **21**:85–92.
16. **Tanaka, S. K., R. A. S. Summerill, B. F. Minassian, K. Bush, D. A. Visnic, D. P. Bonner, and R. B. Sykes.** 1987. In vitro evaluation of tigemonam, a novel oral monobactam. Antimicrob. Agents Chemother. **31**:219–225.
17. **Waterworth, P., and A. M. Emmerson.** 1979. Dissociated resistance among cephalosporins. Antimicrob. Agents Chemother. **27**:393–398.