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In Vivo Evaluation of Tigemonam, a Novel Oral Monobactam

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Tigemonam, a new monobactam with excellent activity against gram-negative bacteria, was evaluated for in vivo efficacy and absorption after oral administration to laboratory animals. Tigemonam is absorbed when administered orally to mice and dogs. In a variety of gram-negative systemic infections in mice, orally administered tigemonam was efficacious in all infections studied. Comparison drugs such as amoxicillin, cephalexin, and cefaclor were less efficacious, especially in infections caused by β-lactamase-producing organisms. In localized infections, tigemonam also demonstrated excellent in vivo activity. In acute pyelonephritis in mice caused by *Escherichia coli* or *Proteus* sp., tigemonam was very effective. In a rat lung model with *Klebsiella pneumoniae*, tigemonam was active with a median effective dose of 46 mg/kg compared with 160 mg/kg for cefaclor and over 200 mg/kg for amoxicillin. Tigemonam was well absorbed in laboratory animals and with its excellent gram-negative spectrum of activity should prove of value in oral antibiotic therapy in humans.

The discovery of the monobactams, a novel class of monocyclic β-lactam antibiotics, led to the development of aztreonam as an injectable antibacterial agent with potent and specific activity against aerobic gram-negative bacteria (1, 7). Chemical manipulation of the monobactam nucleus has resulted in the synthesis of a new monobactam, tigemonam, with excellent in vitro activity against members of the family *Enterobacteriaceae*, *Haemophilus influenzae*, and *Neisseria gonorrhoeae* as well as good β-lactamase stability as reported by Tanaka et al. (8). In contrast to aztreonam, however, tigemonam is absorbed in laboratory animals when administered orally.

We have studied the absorption and pharmacokinetic profile of orally administered tigemonam in mice and dogs. In addition, the therapeutic potential of tigemonam and selected oral antibiotics was determined in a variety of systemic and localized gram-negative bacterial infections in laboratory animals.

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MATERIALS AND METHODS

Antibacterial agents. Tigemonam and amoxicillin were prepared by E. R. Squibb & Sons (Princeton, N.J.). The other reference antibacterial agents were obtained from the following companies: cephalexin and cefaclor from Eli Lilly & Co. (Indianapolis, Ind.); clavulanic acid from Beecham Laboratories, Inc. (Bristol, Tenn.); enoxacin from Warner-Lambert Co. (Ann Arbor, Mich.); and norfloxacin from Merck Sharp & Dohme (Rahway, N.J.).

Animals. Female CD-1 mice (20 to 22 g) were obtained from Charles River Breeding Laboratories, Kingston, N.Y. Female Swiss-Webster mice (18 to 20 g) and Sprague Dawley rats (125 to 150 g) were from Taconic Farms, Germantown, N.Y. Animals were maintained on Purina Rodent Chow, Ralston Purina Co., and water ad libitum.

Male beagle dogs (10 kg) from White Eagle, Doylestown, Pa., were also used for pharmacokinetic studies.

Pharmacokinetic studies. Food was removed from animals used for pharmacokinetic studies 16 h before the test compound was administered. For serum studies, mice (four per route of administration) were exsanguinated, and dogs (four per route) were bled from the femoral vein at specific time intervals after the administration of the compound.

Both sera and urine were assayed by a conventional agar-well technique. Serum samples were extracted with a equal volume of acetonitrile to precipitate serum proteins before assay. Samples were mixed and centrifuged at 1,000 × *g* for 10 min, and the supernatants were collected for bioassay. The assay organism for tigemonam was *Escherichia coli* SC 12155 with a quantitative detection limit of 0.5 μg/ml. The assay organism for amoxicillin, cephalexin, and cefaclor was *Micrococcus luteus* SC 2495 with a detection limit of 0.1 μg/ml for these antibiotics.

Dog serum samples were analyzed by high-pressure liquid chromatography (HPLC) as well as by bioassay. The HPLC components included two M6000 pumps controlled by a model 660 solvent controller (Waters Associates Inc., Milford, Mass.); a Hewlett-Packard 3390A integrator (Hewlett-Packard Co., Palo Alto, Calif.); an ISS autosampler and LC-75 spectrophotometric detector (The Perkin-Elmer Corp., Norwalk, Conn.) set to a wavelength of 293 nm. The mobile phase was acetonitrile (25%; Burdick and Jackson) and 0.005 M tetrabutylammonium hydrogen sulfate, pH 3 (75%; Aldrich Chemical Co., Inc., Milwaukee, Wis.), delivered at 1.5 ml/min. A reverse-phase C_{18} (octadecyl, 5-μm), 4.5- by 250-mm column (IBM Instruments) was used in the analysis of the samples. Retention time for tigemonam was 7.6 min in this system. Samples for HPLC analysis were extracted with acetonitrile and centrifuged like the samples for bioassay. The HPLC and bioassay methods for tigemonam had a correlation coefficient of 0.97.

Susceptibility testing. MICs were determined by agar dilution testing. Enterobacteria were prepared from overnight broth cultures diluted to ca. 5×10^8 CFU/ml, and 0.001 ml (5×10^5 CFU) was delivered to agar plates (Diagnostic Sensi-

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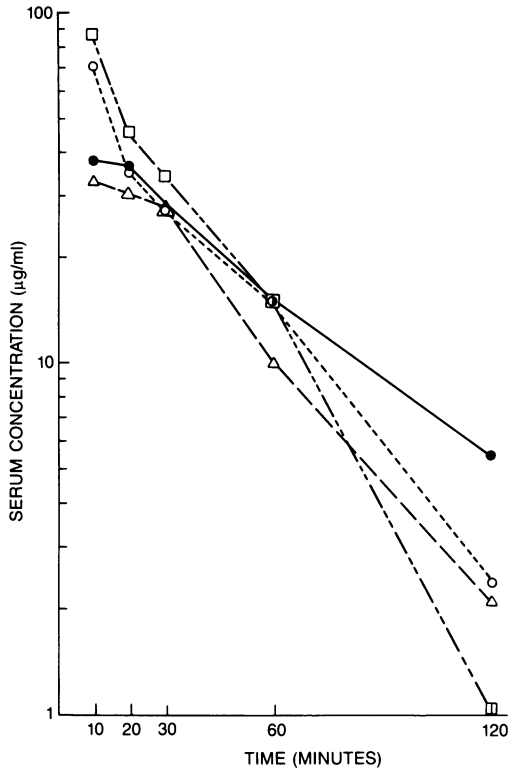

FIG. 1. Comparative pharmacokinetics in mouse serum of tigemonam (●), cefaclor (○), cephalexin (□), and amoxicillin (△) given orally at a dose of 50 mg/kg.

tivity Test Agar; Oxoid Ltd., London, England) containing twofold serial dilutions of the test antibiotic. A standard inoculum of 5×10^5 CFU was used, because it differentiates moderately β-lactamase-stable β-lactam antibiotics from highly stable compounds. *Haemophilus influenzae* was grown on fresh chocolate agar plates and suspended in broth to yield ca. 5×10^8 CFU/ml; 0.001 ml (5×10^5 CFU) was delivered to proteose agar no. 3 (Difco Laboratories, Detroit, Mich.) supplemented with 1% hemoglobin (BBL Microbiology Systems, Cockeysville, Md.) and 1% IsoVitaleX (BBL) containing the test antibiotic (8). Plates were incubated under proper atmospheric conditions at 37°C, and MICs were determined at 24 h as the minimal concentration of antibiotic completely inhibiting growth.

Mouse systemic infections. Mice were infected via the intraperitoneal route with the test pathogen contained in 1.0 ml of 5% hog gastric mucin (American Laboratories, Omaha, Neb.). Treatment was administered by the oral route in divided doses 1 and 5 h after infection. At least three levels of the test compounds were employed with 10 mice at each level. The median effective dose (ED_{50}) was calculated from the number of animals surviving the 6-day observation period by the method of Reed and Muench (5).

Urinary tract infections. Diuresis was induced in mice for 7 days by replacing the drinking water with a 5% glucose solution and reducing food intake to 1 g of mouse chow per mouse per day. Organisms were grown overnight in brain heart infusion broth at 37°C with gentle shaking and then subcultured into fresh brain heart infusion broth for an additional 4 h incubation before use. Then the animals were infected by direct injection into the bladder of 50 μl of the 4-h culture or a dilution of the 4-h culture in brain heart infusion broth, depending on the organism. This procedure, developed by Keene and Freedman (4), results in a reproducible acute ascending pyelonephritis in infected animals. Bacteria used in the model of acute pyelonephritis were *E. coli* SC 12677 (Yale strain, β-lactamase negative), *E. coli* SC 12199 (β-lactamase positive), *Proteus mirabilis* SC 9575, and *P. vulgaris* SC 11210. Animals were maintained on the restricted food intake and 5% glucose water regimen for the duration of the experiment.

Groups of 10 mice were treated with the appropriate antibiotic in varied concentrations beginning 5 to 7 days postinfection. Antibiotics were given as three oral doses per day spaced 3.5 to 4 h apart for 2 consecutive days. Mice were sacrificed 15 h after the last treatment; their kidneys were removed, homogenized in 5 ml of sterile water, serially diluted in water, and then plated on MacConkey's agar to obtain bacterial counts. The number of bacteria was expressed as $\log_{10}$ CFU present in both kidneys. The least number of bacteria detected by this procedure was 25 CFU per pair of kidneys.

The ED_{50} was defined as the amount of antibiotic required to reduce the viable bacterial count in the kidneys by 2 $\log_{10}$ units compared with the number of kidney CFUs obtained from untreated infected animals. The ED_{50} for each antibiotic was calculated by the method of Reed and Muench (5).

Rat lung infection. A lung infection was initiated in rats by the method of Cash et al. (3). *Klebsiella pneumoniae* SC 12330 from an overnight culture grown in brain heart infusion broth at 37°C with shaking was entrapped in microagarose beads prepared with agarose (Bio-Rad Laboratories, Richmond, Calif.) and injected intratracheally. Each animal received 100 beads containing a total of ca. 10^3 CFU of the organism. Animals (10 rats per group) were treated orally three times a day, 3 to 4 h apart, for 2 days begining at 1 h postinfection. Rats were sacrificed 15 h after the last treatment, the lungs were removed and homogenized in 5 ml of distilled water, and appropriate dilutions of the homogenate were plated on MacConkey agar to determine the number of CFU present in each animal's lungs. The least number of bacteria detected by this procedure was 25 CFU per pair of lungs. ED_{50}s were calculated based on a 2-$\log_{10}$ reduction in

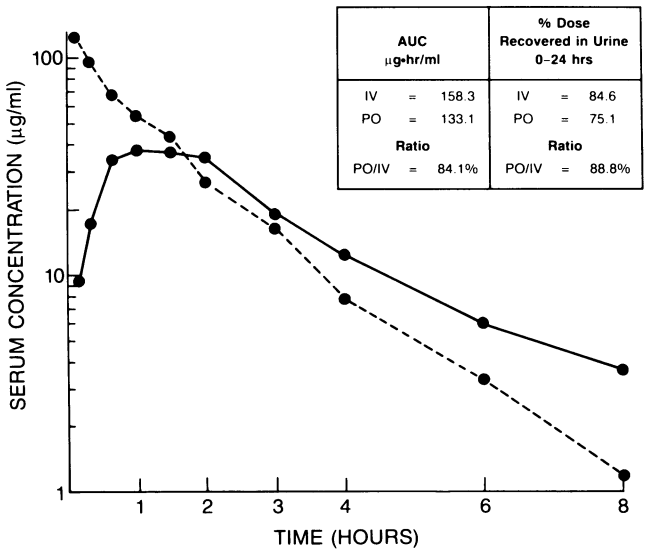

FIG. 2. Pharmacokinetics in dog serum of tigemonam given orally (●——●) or intravenously (●– – –●) at a dose of 25 mg/kg.

CFU present in treated lungs compared with the number of CFU present in the lungs of infected, untreated rats by the method of Reed and Muench (5).

RESULTS

Pharmacokinetics in experimental animals. The levels of tigemonam and several other oral antibiotics in the serum of mice are noted in Fig. 1. In mice peak levels serum of 38 μg/ml were obtained 10 min after oral administration of 50 mg/kg. The levels of tigemonam in serum were comparable to those of cefaclor, cephalexin, and amoxicillin in mice. Tigemonam had a higher level in serum at 120 min than did the other antibiotics employed.

In dogs, excellent absorption was noted with peak levels in serum of 38.1 μg/ml obtained 1 h after oral administration of 25 mg/kg (Fig. 2). In addition, 75% of the administered dose was recovered in the urine, yielding a bioavailability of about 85%. For dogs, determinations of tigemonam by bioassay and HPLC were in very good agreement.

TABLE 1. Efficacy of tigemonam and other oral antibiotics in systemic murine infections

Organism	Infectious dose, CFU (xLD_{50})[a]	Test compound	MIC (μg/ml)	ED_{50} (mg/kg)
Escherichia coli SC 8294	5.3×10^5 (>100)	Tigemonam	0.4	1.4
		Amoxicillin	3.1	9.8
		Cephalexin	12.5	10.9
		Cefaclor	1.6	1.5
		Norfloxacin	0.1	2.3
		Enoxacin	0.4	1.8
E. coli SC 12199	3.5×10^3 (>1,000)	Tigemonam	0.2	1.5
		Amoxicillin	>100	>200
		Cephalexin	12.5	22.1
		Cefaclor	12.5	3.8
		Norfloxacin	0.1	3.5
		Enoxacin	0.2	6.3
Salmonella schottmulleri SC 3850	1.7×10^3 (>100)	Tigemonam	0.4	0.7
		Amoxicillin	0.2	1.5
		Cephalexin	3.1	6.0
		Cefaclor	0.4	0.5
		Norfloxacin	0.1	2.6
		Enoxacin	0.2	1.8
Proteus mirabilis SC 9575	5.9×10^4 (100)	Tigemonam	0.1	0.3
		Amoxicillin	1.6	3.5
		Cephalexin	25.0	37.1
		Cefaclor	6.3	0.8
		Norfloxacin	0.1	2.5
		Enoxacin	1.6	6.3
Providencia rettgeri SC 8217	8.0×10^5 (35)	Tigemonam	<0.05	0.2
		Amoxicillin	>100	>200
		Cephalexin	>100	>200
		Cefaclor	100	>200
		Norfloxacin	0.4	5.4
		Enoxacin	0.8	>12.5
Klebsiella pneumoniae SC 12216	3.0×10^3 (>100)	Tigemonam	0.8	0.9
		Amoxicillin	>100	>200
		Cephalexin	6.3	8.1
		Cefaclor	6.3	1.5
		Norfloxacin	0.2	2.0
		Enoxacin	0.8	2.0
Serratia marcescens SC 9782	2.3×10^2 (32)	Tigemonam	0.4	0.5
		Amoxicillin	>100	>200
		Cephalexin	>100	>200
		Cefaclor	>100	>200
		Norfloxacin	0.2	2.7
		Enoxacin	0.4	1.8
Enterobacter cloacae SC 11078	6.1×10^2 (265)	Tigemonam	3.1	3.9
		Amoxicillin	>100	>200
		Cephalexin	>100	>200
		Cefaclor	>100	>200
		Norfloxacin	0.1	2.7
		Enoxacin	0.8	3.1
Haemophilus influenzae SC 10556	3.0×10^6 (214)	Tigemonam	<0.05	1.8
		Amoxicillin	>100	38.6
		Cephalexin	12.5	9.7
		Cefaclor	6.3	1.1
		Norfloxacin	<0.05	>25
		Enoxacin	0.4	11.7

[a] LD_{50}, 50% lethal dose.

TABLE 2. Efficacy of tigemonam in acute pyelonephritis caused by *E. coli*[a] in mice

Organism	Test compound	MIC (μg/ml)	ED_{50} (mg/kg per day)
E. coli SC 12677[b] (β-lactamase negative)	Tigemonam	0.4	1.0
	Amoxicillin	3.1	3.9
	Cephalexin	12.5	9.7
	Cefaclor	12.5	3.1
E. coli SC 12199[c] (β-lactamase positive)	Tigemonam	0.2	0.5
	Amoxicillin	>100	>200
	Amoxicillin-clavulanic acid (2:1)	6.3	>50[d]
	Cephalexin	12.5	19.8
	Cefaclor	100	10.0

[a] Results are averages of three experiments.
[b] A total of 4×10^5 CFUs were present in untreated infected kidneys at time of culture.
[c] A total of 3×10^6 CFUs were present in untreated infected kidneys at time of culture.
[d] Based on amoxicillin.

Efficacy of tigemonam in mouse systemic infections. The relative efficacies of tigemonam and other orally administered antibiotics were determined in gram-negative bacterial systemic infections in mice (Table 1). In general, tigemonam was the most potent compound tested. The superiority of tigemonam compared with other oral β-lactam antibiotics was most evident when the pathogen was a β-lactamase producer, as in the case of *E. coli* SC 12199 or *Providencia*, *Klebsiella*, *Serratia*, and *Enterobacter* species. When compared with two quinolones, tigemonam was also superior. For example, despite good in vitro activity, norfloxacin (*E. coli* SC 12199, *Haemophilus influenzae*) and enoxacin (*E. coli* SC 12199, *Providencia* sp.) were not as efficacious as tigemonam in vivo.

Urinary tract infections in mice. Several gram-negative bacteria associated with urinary tract infections in humans were used in the mouse model of acute pyelonephritis. These infections were severe as indicated by the high number of organisms recovered (10^4 to 10^6 CFU) and the presence of noticeable abscesses on many infected kidneys removed from untreated control animals. In the two *E. coli* infections (Table 2) tigemonam was highly efficacious in reducing the bacterial load in the kidneys. In the infection caused by the β-lactamase-producing *E. coli* SC 12199, tigemonam retained its efficacy, whereas amoxicillin was rendered ineffective. The addition of the β-lactamase inhibitor clavulanic acid to amoxicillin, although effective in vitro (Table 2), did not appear to be effective in maintaining the activity of amoxicillin in the animal.

In infections caused by *P. mirabilis* and *P. vulgaris*, tigemonam was also superior to the other antibiotics tested, with ED_{50}s of 1 and 3.2 mg/kg, respectively (Table 3).

TABLE 3. Efficacy of tigemonam in acute pyelonephritis caused by *Proteus* spp. in mice[a]

Organism	Test compound	MIC (μg/ml)	ED_{50} (mg/kg per day)
P. mirabilis[b] SC 9575	Tigemonam	0.1	1.0
	Amoxicillin	1.6	11.3
	Cephalexin	25	>100
	Cefaclor	6.3	21.6
P. vulgaris[c] SC 11,210	Tigemonam	<0.05	3.2
	Amoxicillin	>100	54.6
	Cephalexin	>100	25.0
	Cefaclor	>100	4.5

[a] Results are averages of three experiments.
[b] A total of 10^6 CFUs were present in untreated infected kidneys at time of culture.
[c] A total of 1.5×10^4 CFUs were present in untreated infected kidneys at time of culture.

Efficacy of tigemonam in a rat lung infection. The lungs of rats infected intratracheally with *K. pneumoniae* in agarose beads contained approximately 10^6 CFU of the organism when cultured at the end of the experiment. Tigemonam administered three times a day for 2 days was efficacious in reducing the bacterial load in the lungs as indicated by an ED_{50} of 46 mg/kg per day (Table 4). The only other compound tested that demonstrated any efficacy in this model was cefaclor, which had an ED_{50} of 160 mg/kg per day, about fourfold higher than tigemonam. Cephalexin and amoxicillin were ineffective, with ED_{50} values over 200 mg/kg per day. As in the case of the kidney infections in mice, the addition of clavulanic acid to amoxicillin did not improve the efficacy of amoxicillin in this lung infection.

DISCUSSION

The monobactam tigemonam was absorbed after oral administration in mice and dogs, giving levels in serum that were comparable to those of other oral antibiotics in mice.

It is interesting to note the outstanding absorption of orally administered tigemonam in dogs. The new oral cephalosporin cefixime (FK027) is well absorbed in dogs and apparently has a somewhat extended half-life (6). However, if one compares the bioavailability of tigemonam in dogs to that of cefixime by using serum area under the curve (AUC) values [(peroral AUC/intravenous AUC) × 100], cefixime has a bioavailability of 47% compared with 84% for tigemonam.

In a variety of infections in laboratory animals, ranging from systemic infections in mice to lung infections in rats, tigemonam was highly efficacious, being superior to the other oral compounds tested. Only the two quinolones tested, norfloxacin and enoxacin, approached the broad gram-negative efficacy of tigemonam in the systemic model infections employed in this study. For example in systemic infections in mice caused by *Serratia marcescens* and *Providencia rettgeri* only norfloxacin approached the in vivo efficacy of tigemonam.

The failure of amoxicillin and clavulanic acid in the infections caused by β-lactamase-producing organisms was surprising. Clavulanic acid has been reported to be effective in protecting amoxicillin in a variety of model infections involving β-lactamase-producing bacteria (2) and has been shown to penetrate tissues as well as amoxicillin. The reason for the ineffectiveness of clavulanic acid-amoxicillin in the pyelonephritis and lung model is unknown. It may be that in these models the clavulanic acid is not penetrating to the site of infection at the same rate as amoxicillin or is not fully bioavailable. Extended use in the clinic will be needed to establish the true merit of this combination.

TABLE 4. Efficacy of tigemonam in a rat lung infection caused by *K. pneumoniae*[a]

Test compound	MIC (mg/ml)	ED_{50} (mg/kg per day)
Tigemonam	0.4	46.2
Cefaclor	3.1	160.4
Cephalexin	12.5	>200
Amoxicillin	>100	>200
Amoxicillin-clavulanic acid (2:1)	1.6	>200[b]

[a] A total of 8×10^5 CFUs were present in untreated infected lungs at time of culture. Results are averages of three experiments.
[b] Based on amoxicillin.

With its excellent pharmacokinetic profile, in vivo efficacy in experimental infections, improved spectrum of gram-negative activity, and β-lactamase stability, tigemonam should be evaluated as a therapeutic agent in gram-negative infections in humans, particularly in infections of the urinary and pulmonary tracts.

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