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In Vitro Comparison of *N*-Formimidoyl Thienamycin, Piperacillin, Cefotaxime, and Cefoperazone

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The antibacterial activity of *N*-formimidoyl thienamycin was compared with those of cefotaxime, cefoperazone, and piperacillin against 536 clinical aerobic isolates.

Thienamycin is a new β-lactam antibiotic produced by *Streptomyces cattleya* (6). The parent compound, thienamycin, is highly active against anaerobic bacteria, *Pseudomonas aeruginosa*, and staphylococci (7, 10, 15). We report a comparison of stabilized thienamycin with three new β-lactam antibiotics: piperacillin, cefoperazone, and cefotaxime.

All bacteria were clinical isolates. Four antibiotics were tested. *N*-Formimidoyl thienamycin (MK0787) was supplied by Merck & Co., Inc., Rahway, N.J.; cefotaxime (920 μg/mg) was from Hoechst-Roussel Pharmaceuticals, Inc., Somerville, N.J.; piperacillin (911 μg/mg) was from Lederle Laboratories, Pearl River, N.Y.; and cefoperazone (936 μg/mg) was from Pfizer Inc., New York, N.Y.

Stock solutions of the antibiotics were prepared at 1,000 μg/ml in the following diluents: *N*-formimidoyl thienamycin in potassium phosphate buffer (pH 7.0); piperacillin in potassium phosphate buffer (pH 6.0); cefotaxime and cefoperazone in distilled water.

A semiautomated broth microdilution method was used, employing a Dynatech MIC-2000 device. Mueller-Hinton broth was supplemented with 55 mg of calcium chloride per liter and 24 mg of magnesium chloride per liter. An inoculum was prepared by transferring three to four isolated colonies to Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) and incubating at 35°C for 2 to 4 h until turbidity was equivalent to 0.5 $BaSO_4$ turbidity standard (14). An inoculum was prepared which yielded either 10^5 or 10^3 colony-forming units (CFU) per well in the test plate. Plates were incubated at 35°C for 18 to 24 h. The lowest concentration showing no visible growth in a well was recorded as the minimal inhibitory concentration (MIC). The minimal bactericidal concentration (MBC) was determined by using the MIC-2000 inoculator to subculture 0.0015 ml from each well to a Mueller-Hinton agar plate. The MBC was recorded as the lowest concentration that gave no growth on subculture after incubation at 35°C for 18 h (12). The concentration needed to inhibit 50 or 90% of strains was computer generated, using a probit analysis of the MICs for each antibiotic. The concentration ranges were derived from published data for piperacillin, cefotaxime, cefoperazone, and thienamycin (1-3, 11). Twofold serial dilutions were used for all antibiotics. The concentration ranges were (μg/ml): cefotaxime, 0.01 to 50; cefoperazone, 0.05 to 50; piperacillin, 0.2 to 50; *N*-formimidoyl thienamycin, 0.01 to 64.

The susceptibilities of *Staphylococcus aureus* (ATCC 25923), *P. aeruginosa* (ATCC 27853), and *Escherichia coli* (ATCC 25922) were determined for all test antibiotics with each group of clinical isolates tested.

Table 1 summarizes the results obtained for the four test antibiotics for 15 groups of bacteria. *N*-Formimidoyl thienamycin was the most potent of the test agents against *Acinetobacter* sp., *Citrobacter* sp., *Enterobacter* sp., enterococci, *P. aeruginosa*, *Pseudomonas* sp. (other than *P. aeruginosa*), *Serratia* sp., *S. aureus*, and *Staphylococcus epidermidis*. Cefotaxime was the most potent against *E. coli*, *Klebsiella* sp., *Proteus mirabilis*, *Proteus vulgaris*, and *Providencia* sp. *N*-Formimidoyl thienamycin also inhibited these strains at 2 μg/ml or less. For some species, such as *Acinetobacter* sp., *P. aeruginosa*, and *Pseudomonas* sp. (other than *P. aeruginosa*), *N*-formimidoyl thienamycin was from 20 to 70 times more potent than cefotaxime. Piperacillin at 1.6 μg/ml inhibited only 58.5% of enterococci tested. The highest MICs for *N*-formimidoyl thienamycin were recorded against *P. vulgaris* and *Morganella morganii* (indole-positive *Proteus* sp.) and *Pseudomonas* sp. (other than *P. aeruginosa*). *N*-Formimidoyl thienamycin was the most potent against other *Pseudomonas* species, however. *N*-Formimidoyl thienamycin was 70-fold more potent than cefotaxime against *S. aureus* and 80 times more potent than cefotaxime against *S. epidermidis*. There was no

TABLE 1. *Comparison of cefotaxime, cefoperazone, piperacillin, and thienamycin*[a]

Organism (no. tested)	Antibiotic	MIC[b] (μg/ml)		Range
		For 50% of strains	For 90% of strains	
Acinetobacter sp. (10)	Cefotaxime	3.30	15.00	0.40–25.00
	Cefoperazone	9.70	47.50	1.60–50.00
	Piperacillin	3.90	17.30	0.80–50.00
	Thienamycin	0.07	0.21	0.01–1.00
Citrobacter sp. (15)	Cefotaxime	0.30	14.20	0.025–50.00
	Cefoperazone	0.40	7.10	0.05–50.00
	Piperacillin	4.30	22.50	1.60–50.00
	Thienamycin	0.30	0.70	0.10–1.00
Enterobacter sp. (55)	Cefotaxime	0.14	1.60	0.01–50.00
	Cefoperazone	0.30	4.50	0.05–50.00
	Piperacillin	2.00	17.80	0.40–50.00
	Thienamycin	0.50	1.10	0.10–4.00
Escherichia coli (50)	Cefotaxime	0.03	0.05	0.01–0.20
	Cefoperazone	0.14	1.90	0.025–6.40
	Piperacillin	1.90	9.80	0.40–50.00
	Thienamycin	0.12	0.24	0.05–1.00
Enterococcus sp. (55)	Cefotaxime	≥50.00		≥50.00
	Cefoperazone	≥50.00		25.00–50.00
	Piperacillin	5.70	21.50	3.20–25.00
	Thienamycin	2.00	2.00	2.00
Klebsiella sp. (55)	Cefotaxime	0.01	0.06	0.025–0.80
	Cefoperazone	0.20	1.30	0.05–12.50
	Piperacillin	3.70	15.00	1.60–50.00
	Thienamycin	0.10	0.23	0.10–0.50
Morganella sp. (22)	Cefotaxime	0.05	5.30	0.01–25.00
	Cefoperazone	1.50	4.60	0.80–50.00
	Piperacillin	0.60	3.20	0.40–50.00
	Thienamycin	2.20	3.40	1.00–4.00
Proteus mirabilis (53)	Cefotaxime	0.01	0.02	0.01–0.05
	Cefoperazone	0.50	0.90	0.20–12.50
	Piperacillin	≤0.40	≤0.40	0.40–50.00
	Thienamycin	0.90	1.90	0.25–4.00
P. vulgaris (13)	Cefotaxime	0.02	0.05	0.01–6.40
	Cefoperazone	0.80	7.00	0.05–12.50
	Piperacillin	0.30	9.80	0.40–50.00
	Thienamycin	1.60	3.70	0.50–4.00
Pseudomonas aeruginosa (53)	Cefotaxime	5.20	21.30	0.20–50.00
	Cefoperazone	2.50	12.60	0.10–50.00
	Piperacillin	3.20	10.00	0.40–50.00
	Thienamycin	0.60	1.10	0.50–4.00
Other *Pseudomonas* spp. (18)	Cefotaxime	4.00	≥50.00	0.10–50.00
	Cefoperazone	3.60	25.60	0.20–50.00
	Piperacillin	5.10	≥50.00	0.40–50.00
	Thienamycin	0.60	4.40	0.05–64.00
Providencia sp. (18)	Cefotaxime	0.03	0.50	0.01–3.20
	Cefoperazone	1.30	9.50	0.10–25.00
	Piperacillin	2.50	29.00	0.40–50.00
	Thienamycin	0.70	1.50	0.25–2.00

TABLE 1—*Continued*

Organism (no. tested)	Antibiotic	MIC[b] (μg/ml)		Range
		For 50% of strains	For 90% of strains	
Serratia sp. (42)	Cefotaxime	0.20	4.80	0.05–50.00
	Cefoperazone	1.90	17.20	0.20–50.00
	Piperacillin	3.60	36.50	0.40–50.00
	Thienamycin	0.40	0.80	0.25–2.00
Staphylococcus aureus (49)	Cefotaxime	1.00	1.30	0.80–3.20
	Cefoperazone	1.60	2.80	1.60–6.40
	Piperacillin	6.20	40.50	0.80–50.00
	Thienamycin	0.015	0.018	0.01–0.025
S. epidermidis (28)	Cefotaxime	0.80	4.80	0.10–50.00
	Cefoperazone	1.70	10.40	0.20–50.00
	Piperacillin	1.50	9.00	0.40–50.00
	Thienamycin	0.01	0.30	0.01–16.00

[a] Thienamycin, *N*-Formimidoyl thienamycin.
[b] 10^5 CFU/ml.

significant inoculum effect on MICs for cefoperazone or *N*-formimidoyl thienamycin.

There were greater than fourfold differences between the MICs at 10^3 and 10^5 CFU when cefotaxime was tested against *M. morganii*, *Citrobacter* sp., and *Serratia* sp. (Table 2). An inoculum effect was also observed for piperacillin when tested against *S. aureus*, *P. vulgaris*, and enterococci. At 10^5 CFU per well, piperacillin was bactericidal at its MIC, as has been previously reported (13).

The MICs and MBCs for *N*-formimidoyl thienamycin recorded here compare closely to those previously reported (4, 8). The MICs for *Citrobacter* sp. and *P. vulgaris* were higher than those reported by Kesado and co-workers (7). Compared with those studies in which thienamycin rather than *N*-formimidoyl thienamycin was used, the MICs with the stabilized form were consistently lower (10, 15). The *E. coli* strains in this study were more susceptible to piperacillin than those in the study by Fu and Neu (1). Otherwise, our results for piperacillin were similar to those of other reported studies (16). The results for cefotaxime were similar to those of Masuyoshi et al. and of Fuchs et al. (2, 9). The MICs and MBCs recorded for cefoperazone were less than those reported by Hinkle et al. (3) but similar to other studies (5).

The stabilized form of thienamycin is a potent bactericidal agent. The spectrum is wide, including *P. aeruginosa*, enterococci, staphylococci, and most gram-negative aerobic bacteria. Unlike many new β-lactam antibiotics, the potency of *N*-formimidoyl thienamycin against gram-positive cocci is not reduced. Compared with cefotaxime, cefoperazone, and piperacillin, stabilized thienamycin is the most potent against *Pseudomonas* sp., enterococci, staphylococci, and the majority of gram-negative rods tested.

TABLE 2. *Effect of inoculum on MIC[a] for cefotaxime*

Organism	Inoculum size	
	10^3 CFU/ml	10^5 CFU/ml
Citrobacter sp.	3.57	14.2
M. morganii	0.02	5.3
Serratia sp.	0.2	4.8

[a] MIC (μg/ml) necessary to inhibit 90% of strains.

The wide spectrum, low MICs, and favorable relationship between MICs and MBCs make stabilized thienamycin an excellent antibiotic for clinical trials.

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