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N-Formimidoyl Thienamycin (MK0787): In Vitro Study

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N-Formimidoyl thienamycin (MK0787) was compared in vitro with three other β-lactam and two aminoglycoside antibiotics. It was second in activity only to cefotaxime against members of the *Enterobacteriaceae* and to amikacin against *Pseudomonas* species. It was the most active antibiotic against *Staphylococcus aureus*. Resistance (minimal inhibitory concentration, >128 μg/ml) to *N*-formimidoyl thienamycin was not observed.

Thienamycin is a novel β-lactam antibiotic characterized by a broad spectrum of antibacterial activity (1, 6, 7). Unfortunately, the potential usefulness of this compound was compromised by its poor stability (3). This problem now has been solved with the development of the amidine derivative *N*-formimidoyl thienamycin or MK0787 (K. J. Wildonger, W. J. Leanza, T. W. Miller, and B. G. Christensen, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 19th, Boston, Mass., abstr. no. 232, 1979). *N*-formimidoyl thienamycin already has been shown to be uniformly active against a number of aerobic and anaerobic bacteria resistant to other β-lactam antibiotics (4), as well as to be at least 10-fold more active in vivo than cephalothin, cefazolin, ampicillin, and cefoxitin against both gram-positive and gram-negative organisms (5).

The purpose of this study was to further evaluate the in vitro activity of *N*-formimidoyl thienamycin and to compare this drug with a variety of other antimicrobial agents, including both β-lactam (cefotaxime, cefoxitin, and cefamandole) and aminoglycoside (gentamicin and amikacin) antibiotics. Test organisms included 108 clinical isolates of aerobic pathogens, including many that are resistant to gentamicin.

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A total of six antibiotics were tested. These included: *N*-formimidoyl thienamycin (L638, 596-01D2) and cefoxitin sodium (batch L620, 388-01-B340), furnished by Merck Sharp & Dohme Research Laboratories, Rahway N.J.; amikacin (lot F8653), supplied by Bristol Laboratories, Syracuse, N.Y.; cefamandole lithium (lot S1-88-8F), supplied by Eli Lilly and Co., Indianapolis, Ind.; cefotaxime, or HR 756 (lot RP 2713), supplied by Hoechst-Roussel Pharmaceuticals Inc., Sumerville, N.J.; and gentamicin sulfate (batch GMC 8-M-6059), supplied by the Schering Corp., Kenilworth, N.J.

All drugs were supplied as powdered laboratory standards. Immediately before each series of in vitro tests, stock solutions were prepared in Mueller-Hinton broth and filter sterilized. These then were diluted in Mueller-Hinton broth to provide serial concentrations ranging from 1,280 to 1.25 μg of active drug per ml in 2-ml volumes.

A total of 108 clinical isolates of gram-negative and gram-positive bacterial pathogens were tested. These included *Enterobacter aerogenes* (9 isolates), *Enterobacter cloacae* (10 isolates), *Escherichia coli* (20 isolates), *Klebsiella pneumoniae* (7 isolates), *Proteus* spp. (16 isolates), *Pseudomonas* spp. (16 isolates), *Serratia* spp. (20 isolates), and *Staphylococcus aureus* (10 isolates). Most isolates were obtained from specimens submitted to the clinical laboratories of three Richmond, Va. area hospitals. Identifications of the individual isolates were determined in the respective hospital laboratories. Standard reference strains of *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853), and *S. aureus* (ATCC 25923) were included in all individual experiments.

Minimal inhibitory concentrations (MICs) of the six antibiotics were determined by using the World Health Organization-International Collaborative study agar dilution procedure (2). Square (100-mm) Intergrid petri plates (Falcon Plastics, Oxnard, Calif.) were filled with 20 ml of Mueller-Hinton agar containing 10-fold dilutions of the antibiotic solutions described above; thus, final test concentrations ranged from 128 to 0.125 μg/ml. Inocula were prepared by growing individual strains overnight in Mueller-Hinton broth; these cultures were adjusted turbidimetrically to contain approximately 10^7 colony-forming units per ml. A replicator device was used to inoculate drug-containing and drug-free

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TABLE 1. *Comparative in vitro activities of MK0787 and five other antibiotics as measured against 108 routine aerobic clinical bacterial isolates*[a]

Organism (no. tested)	Antibiotic	MIC (μg/ml)[b]			
		Range	G-MIC	MIC_{50}	MIC_{90}
Gram-negative isolates					
Enterobacter aerogenes (9)	*N*-Formimidoyl thienamycin	0.25–4	0.9	1	4
	Cefamandole	2–≥128	14.8	8	≥128
	Cefotaxime	≤0.125–4	0.3	0.25	4
	Cefoxitin	4–≥128	87.1	128	≥128
	Amikacin	1–4	1.7	2	4
	Gentamicin	0.5–32	2.3	2	32
Enterobacter cloacae (10)	*N*-Formimidoyl thienamycin	0.25–2	0.8	1	1
	Cefamandole	0.5–128	5.7	8	16
	Cefotaxime	≤0.125–4	0.3	≤0.125	0.5
	Cefoxitin	4–≥128	59.7	64	≥128
	Amikacin	2–4	1.4	2	4
	Gentamicin	1–32	2.6	2	4
Escherichia coli (20)	*N*-Formimidoyl thienamycin	0.25–1	0.4	0.5	1
	Cefamandole	1–≥128	7.7	4	≥128
	Cefotaxime	≤0.125–1	0.2	≤0.125	0.25
	Cefoxitin	1–≥128	3.4	2	8
	Amikacin	0.5–16	1.6	2	4
	Gentamicin	2–≥128	11.3	4	128
Klebsiella pneumoniae (7)	*N*-Formimidoyl thienamycin	0.25–0.5	0.3	0.25	0.5
	Cefamandole	8–≥128	35.3	32	≥128
	Cefotaxime	≤0.125	≤0.125	≤0.125	≤0.125
	Cefoxitin	0–2	1.6	2	2
	Amikacin	0.5–2	1.0	1	2
	Gentamicin	64–128	95.1	128	128
Proteus spp. (16)	*N*-Formimidoyl thienamycin	0.5–4	1.2	1	2
	Cefamandole	0.5–≥128	7.0	32	≥128
	Cefotaxime	≤0.125–4	0.13	≥0.125	2
	Cefoxitin	2–16	3.7	4	8
	Amikacin	0.5–16	2.1	2	8
	Gentamicin	1–8	3.0	4	8
Pseudomonas spp. (16)	*N*-Formimidoyl thienamycin	1–32	3.5	2	16
	Cefamandole	4–≥128	103.1	≥128	≥128
	Cefotaxime	0.5–≥128	12.9	16	≥128
	Cefoxitin	1–≥128	30.6	≥128	≥128
	Amikacin	0.25–16	1.3	1	8
	Gentamicin	4–≥128	17.5	8	≥128
Serratia spp. (20)	*N*-Formimidoyl thienamycin	2–8	3.7	4	8
	Cefamandole	1–≥128	33.1	32	≥128
	Cefotaxime	≤0.125–8	0.5	0.25	4
	Cefoxitin	8–≥128	34.3	16	≥128
	Amikacin	0.5–4	1.8	2	2
	Gentamicin	2–64	18.4	64	64

[a] As determined with the World Health Organization-International Collaborative Study agar dilution procedure.

[b] G-MIC, Geometric mean MIC; MIC_{50} and MIC_{90}, MICs for inhibition of 50 and 90% of isolates tested.

TABLE 1—(*Continued*)

Organism (no. tested)	Antibiotic	MIC (μg/ml)[b]			
		Range	G-MIC	MIC_{50}	MIC_{90}
Gram-positive isolates					
Staphylococcus aureus (10)	*N*-Formimidoyl thienamycin	≤0.125	≤0.125	≤0.125	≤0.125
	Cefamandole	≤0.125–1	0.4	0.25	0.5
	Cefotaxime	1–2	1.5	2	2
	Cefoxitin	2.0	2.0	2	2
	Amikacin	0.25–2	0.9	1	1
	Gentamicin	0.5–1	0.6	0.5	1

control plates; test inocula contained approximately 10^4 organisms. The MIC, determined after 18 h of incubation at 37°C, was defined as the lowest concentration of drug allowing growth of two or less bacterial colonies. A fine growth or haze was ignored. Cumulative percent inhibition and geometric mean MICs were determined for each species and each drug.

Results of these studies are presented in Table 1. Among the β-lactam-type antibiotics, *N*-formimidoyl thienamycin and cefotaxime generally were the most active compounds when tested against members of the *Enterobacteriaceae*. Most isolates of *E. aerogenes*, *E. cloacae*, *E. coli*, *K. pneumoniae*, and *Proteus* spp. were inhibited by 1.0 μg or less of either *N*-formimidoyl thienamycin or cefotaxime per ml. None of the isolates was resistant to either drug. Cefamandole was inhibitory for most of these same organisms at concentrations ranging from 0.5 to 64 μg/ml. However, two isolates each of *E. aerogenes*, *K. pneumoniae*, and *Proteus* spp., three of *E. coli*, and one of *E. cloacae* were resistant to cefamandole at 128 μg/ml. Most isolates of *E. aerogenes* and *E. cloacae* were resistant to cefoxitin at concentrations of <32 μg/ml; one isolate only of each was inhibited at 4 μg/ml. Cefoxitin was inhibitory for isolates of *E. coli*, *K. pneumoniae*, and *Proteus* spp. at concentrations ranging from 1 to 16 μg/ml. One isolate of *E. coli* was resistant to cefoxitin. Cefotaxime was the most active of the β-lactam-type antibiotics against *Serratia* spp.; MICs ranged from 0.125 to 8 μg/ml, with inhibition of 50% of the isolates at 0.25 μg/ml. *N*-formimidoyl thienamycin was inhibitory between 2 and 8 μg/ml, with inhibition of 50% of the isolates at 4 μg/ml. Neither cefamandole nor cefoxitin was remarkably active against most *Serratia* spp.

N-formimidoyl thienamycin was the most active β-lactam antibiotic against *Pseudomonas* spp. Inhibitory concentrations ranged between 1 and 32 μg/ml, with inhibition of 50% of the isolates occurring at 2 μg/ml. Cefotaxime was less active, with inhibition of only 2 of 16 isolates at 2 μg/ml; inhibition of 50% of the isolates occurred at 16 μg/ml. Two isolates of *Pseudomonas* spp. were totally resistant to cefotaxime. Cefoxitin inhibited six isolates at between 1 and 8 μg/ml, with the remaining 10 isolates being totally resistant. Cefamandole was the least active against *Pseudomonas* spp., with a concentration of 128 μg/ml being required to inhibit 15 of the isolates; one isolate was inhibited at 4 μg/ml.

Amikacin was the most active of the two aminoglycosides tested against the above organisms. Most of the gram-negative isolates were susceptible to 4 μg or less of amikacin per ml; nearly half were clinically resistant (MIC, >4 μg/ml) to gentamicin.

N-Formimidoyl thienamycin was by far the most active antibiotic when tested against *S. aureus*. All isolates were inhibited at a concentration of ≤0.125 μg/ml. The remaining β-lactams and aminoglycosides inhibited most isolates of *S. aureus*, but at concentrations of between 0.25 and 2 μg/ml.

These studies demonstrated that *N*-formimidoyl thienamycin is a unique β-lactam antibiotic in several ways. First, resistance appears to be rare; in this study it was nonexistent. This lack of resistance has been correlated with the inability of either chromosomal or plasmid-associated β-lactamases to hydrolyze the drug (H. C. Neu and P. Labthavikul, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 20th, New Orleans, La., abstr. no 260, 1980). Second, although *N*-formimidoyl thienamycin possesses a high degree of activity against gram-negative species, it also is highly active against *S. aureus*. Third, and as noted by others, it is highly active in vivo against both gram-negative and gram-positive species (5). All of these properties suggest that *N*-formimidoyl thienamycin could play an important role in the management of clinical infections caused by susceptible organisms.

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