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A Comparison of the Antibacterial Activities of *N*-Formimidoyl Thienamycin (MK0787) with Those of Other Recently Developed β-Lactam Derivatives

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The antibacterial activity of *N*-formimidoyl thienamycin (MK0787) was evaluated in 335 clinical isolates of ampicillin-resistant *Enterobacteriaceae*, 50 *Pseudomonas aeruginosa* strains, 28 *Acinetobacter* spp., 50 *Streptococcus faecalis* strains, and 7 oxacillin-resistant *Staphylococcus aureus* strains and was compared with the recently developed β-lactam antibiotics mezlocillin, cefuroxime, cefazedone, cefoperazone, cefotaxime, and moxalactam. Among the gram-negative bacteria, *N*-formimidoyl thienamycin was less active than cefotaxime against *Klebsiella*, *Serratia*, and *Proteus* spp. but had comparable activity against *Escherichia coli* and *Enterobacter* strains. Activity of the thienamycin derivative was somewhat lower than that of moxalactam against most of the strains and superior to that of mezlocillin, cefuroxime, and cefoperazone. Moreover, *N*-formimidoyl thienamycin was the most active drug against *P. aeruginosa* and *Acinetobacter* spp. and had activity comparable to that of ampicillin against *Streptococcus faecalis*. *N*-Formimidoyl thienamycin was bactericidal at concentrations less than twice the minimal inhibitory concentration (MIC) in all gram-negative isolates tested. Oxacillin-resistant staphylococci (MIC of oxacillin, >4 μg/ml) were inhibited at low concentrations of the thienamycin derivative (90% MIC, 0.25 μg/ml); however, *N*-formimidoyl thienamycin was not bactericidal at the 90% MIC. The antibacterial activity of *N*-formimidoyl thienamycin against all of the gram-negative bacilli was observed to be independent of β-lactamase production.

It has been reported recently by several investigators that *N*-formimidoyl thienamycin (MK0787) exhibits a remarkably broad spectrum of antibacterial activity against both gram-negative and gram-positive bacteria (1-3). It was the purpose of this study to compare the antibacterial activities of *N*-formimidoyl thienamycin and other recently developed β-lactam antibiotics against ampicillin-resistant strains of *Enterobacteriaceae*, *Pseudomonas aeruginosa*, *Acinetobacter* spp., *Streptococcus faecalis*, and oxacillin-resistant staphylococci to explore the bactericidal action of *N*-formimidoyl thienamycin against both gram-negative and gram-positive bacteria and to evaluate the effect of β-lactamase production in gram-negative bacilli on the antibacterial activity of *N*-formimidoyl thienamycin.

MATERIALS AND METHODS

Bacterial isolates. Recent isolates from seven hospitals were used in this study; they included 335 strains of ampicillin-resistant *Enterobacteriaceae* (minimal inhibitory concentration [MIC], >16 μg/ml), 50 strains of *P. aeruginosa*, 28 strains of *Acinetobacter* spp., 50 strains of *S. faecalis*, and 7 strains of oxacillin-resistant *Staphylococcus aureus* (MIC, ≥4 μg/ml). All isolates of *Enterobacteriaceae* were identified by the API 20E system (API system S.A., Montalieu-Vercien, France), and all other species were identified by standard procedures (4). All strains were lyophilized before storage.

Antibiotics. Mezlocillin and azlocillin for use in this study were kindly provided by Bayer AG, Leverkusen; ampicillin, oxacillin, cephalothin, cefuroxime, and cefotaxime were provided by Hoechst-Russell Pharmaceuticals, Inc., Frankfurt; cefazedone was provided by Merck & Co., Darmstadt; cefoperazone was provided by Pfizer Inc., Karlsruhe; moxalactam was provided by Eli Lilly & Co., Giessen; and cefoxitin and *N*-formimidoyl thienamycin were provided by Merck Sharp & Dohme, Munich, Germany.

Susceptibility testing. Broth dilution studies were performed in Mueller-Hinton broth (Oxoid Ltd., London, England) with twofold serial dilutions. The inoculum was 5×10^5 colony-forming units per ml. All experiments were carried out in microtiter plates in a final volume of 0.1 ml. The MIC was defined as the lowest concentration of drug which suppressed visible

TABLE 1. MICs for strains used in this study

Organism	No. of isolates + or −[a]	Antimicrobial agent[b]	MIC (μg/ml of culture medium)		
			Range	50%	90%
Escherichia coli	45+	MEZ	2–1,024	128	1,024
		CEFUR	1–256	2	8
		CEFOP	0.125–512	2	256
		CFTX	0.03–2	0.06	1
		MOXAL	0.06–2	0.125	1
		NFT	0.125–4	0.25	0.5
	5−	MEZ	4–1,024	64	1,024
		CEFUR	1–4	2	4
		CEFOP	0.125–4	2	4
		CFTX	0.03–1	0.06	1
		MOXAL	0.06–0.25	0.125	0.25
		NFT	0.25–0.5	0.25	0.5
Klebsiella spp.	28+	MEZ	4–1,024	1,024	1,024
		CEFUR	1–512	4	32
		CEFOP	0.5–1,024	32	1,024
		CFTX	0.03–1	0.03	0.125
		MOXAL	0.06–4	0.25	0.5
		NFT	0.125–1	0.25	1
	22−	MEZ	2–1,024	16	1,024
		CEFUR	1–1,024	2	8
		CEFOP	0.125–1,024	0.25	4
		CFTX	0.03–0.25	0.03	0.125
		MOXAL	0.06–4	0.125	1
		NFT	0.125–4	0.25	1
Enterobacter spp.	29+	MEZ	2–1,024	128	1,024
		CEFUR	1–1,024	128	512
		CEFOP	0.25–1,024	8	512
		CFTX	0.03–128	1	1,280
		MOXAL	0.125–64	2	16
		NFT	0.25–8	1	2
	21−	MEZ	2–16	4	8
		CEFUR	1–32	8	16
		CEFOP	0.125–64	0.5	4
		CFTX	0.03–128	0.5	2
		MOXAL	0.06–8	0.25	0.5
		NFT	0.25–4	1	2
Serratia spp.	23+	MEZ	1–1,024	128	1,024
		CEFUR	32–1,024	128	1,024
		CEFOP	0.5–1,024	32	512
		CFTX	0.25–8	0.5	1
		MOXAL	0.125–8	0.25	1
		NFT	2–8	4	4
	21−	MEZ	1–1,024	8	128
		CEFUR	64–1,024	128	1,024
		CEFOP	0.5–1,024	1	8
		CFTX	0.06–1	0.5	1
		MOXAL	0.125–4	0.25	1
		NFT	2–4	4	4
Citrobacter spp.	30+	MEZ	8–1,024	1,024	1,024
		CEFUR	2–512	8	512

TABLE 1—*Continued*

Organism	No. of isolates + or −[a]	Antimicrobial agent[b]	MIC (μg/ml of culture medium)		
			Range	50%	90%
		CEFOP	0.25–1,024	256	1,024
		CFTX	0.03–128	0.5	64
		MOXAL	0.06–128	0.5	16
		NFT	0.25–2	2	2
	13⁻	MEZ	1–1,024	16	64
		CEFUR	2–512	4	8
		CEFOP	0.125–512	0.5	32
		CFTX	0.03–16	0.06	4
		MOXAL	0.06–32	0.125	8
		NFT	0.125–2	0.25	2
Proteus mirabilis	20⁺	MEZ	2–1,024	1,024	1,024
		CEFUR	1–1,024	2	8
		CEFOP	1–1,024	1,024	1,024
		CFTX	0.03–0.25	0.03	0.25
		MOXAL	0.06–2	0.125	0.5
		NFT	0.125–0.5	4	8
	21⁻	MEZ	1–1,024	2	1,024
		CEFUR	1–1,024	2	1,024
		CEFOP	0.25–1,024	1	128
		CFTX	0.03–128	0.03	0.25
		MOXAL	0.06–1	0.125	0.5
		NFT	2–16	4	8
Proteus vulgaris	3⁺	MEZ	4–1,024	4	1,024
		CEFUR	1–1,024	1,024	1,024
		CEFOP	256–1,024	1,024	1,024
		CFTX	0.03–0.03	0.03	0.03
		MOXAL	0.125–0.25	0.25	0.25
		NFT	8	8	8
	40⁻	MEZ	4–1,024	4	1,024
		CEFUR	2–1,024	512	1,024
		CEFOP	0.5–1,024	1	8
		CFTX	0.03–16	0.06	4
		MOXAL	0.125–1	0.25	0.5
		NFT	1–8	4	8
Morganella morganii	9⁺	MEZ	1–1,024	1,024	1,024
		CEFUR	16–128	64	128
		CEFOP	1–1,024	64	1,024
		CFTX	0.06–16	0.5	16
		MOXAL	0.125–0.5	0.25	0.25
		NFT	4	4	4
	36⁻	MEZ	1–128	2	64
		CEFUR	16–128	32	64
		CEFOP	0.5–1,024	1	8
		CFTX	0.03–8	0.06	2
		MOXAL	0.125–1	0.25	0.5
		NFT	1–8	4	8
Providencia rettgeri	9⁺	MEZ	256–1,024	1,024	1,024
		CEFUR	1–128	16	128
		CEFOP	16–1,024	1,024	1,024
		CFTX	0.06–8	0.25	8

TABLE 1—*Continued*

Organism	No. of isolates + or −[a]	Antimicrobial agent[b]	MIC (μg/ml of culture medium)		
			Range	50%	90%
		MOXAL	0.125–8	0.25	8
		NFT	0.125–8	2	8
	4⁻	MEZ	4–256	16	256
		CEFUR	1–32	2	32
		CEFOP	0.5–4	1	4
		CFTX	0.03–0.25	0.06	0.25
		MOXAL	0.06–1	0.25	1
		NFT	1–8	4	8
Pseudomonas aeruginosa	5⁺	MEZ	16–1,024	1,024	1,024
		CEFUR	NT[c]		
		CEFOP	32–1,024	32	1,024
		CFTX	4–1,024	64	1,024
		MOXAL	2–256	64	256
		NFT	0.5–2	2	2
	45⁻	MEZ	16–1,024	32	256
		CEFUR	NT		
		CEFOP	4–128	8	32
		CFTX	4–128	16	32
		MOXAL	4–256	16	64
		NFT	0.5–8	1	2
Acinetobacter calcoaceticus Lwoffi	9⁻	MEZ	2–128	32	128
		CEFUR	1–1,024	4	1,024
		CEFOP	16–256	32	256
		CFTX	0.25–32	8	32
		MOXAL	4–64	16	64
		NFT	0.03–0.5	0.25	0.5
Acinetobacter calcoaceticus-anitratus	19⁻	MEZ	4–128	16	128
		CEFUR	1–32	8	32
		CEFOP	8–256	32	256
		CFTX	1–32	4	16
		MOXAL	8–256	32	256
		NFT	0.03–0.5	0.25	0.25

[a] +, β-Lactamase positive; −, β-lactamase negative.
[b] MEZ, Mezlocillin; CEFUR, cefuroxime; CEFOP, cefoperazone; CFTX, cefotaxime; MOXAL, moxalactam; NFT, *N*-formimidoyl thienamycin.
[c] NT, Not tested.

growth after incubation at 37°C for 18 h. For determination of the minimal bactericidal concentration (MBC), a sample of 0.01 ml was plated on cystine-lactose-electrolyte-deficient agar (Oxoid). The MBC was defined as the lowest concentration of drug which yielded fewer than five colonies (i.e., 99% kill) after incubation at 37°C for 18 h.

β-Lactamase assay. The presence of β-lactamase was determined in crude cell-free extracts prepared from a 10-ml overnight culture grown in Isosensitest broth (Oxoid). The cells were collected by centrifugation at 7,000 × *g* for 15 min, washed once with distilled water, suspended in 10 ml of distilled water, and subjected to ultrasonic disruption with a Branson B-12 sonifier (Ultrasonic Instruments International, Westbury, N.Y.) for 2.5 min at 4°C. Cell debris was removed by centrifugation at 25,000 × *g* for 20 min. A color change of the chromogenic cephalosporin 87/312 (concentration, 0.5 mg/ml) from yellow to red within 5 min was considered positive (7). The compound 87/312 was kindly supplied by Glaxo Pharmaceuticals Ltd., Greenford, Middlesex, England.

RESULTS

MICs and MBCs against *Enterobacteriaceae*. Two patterns of susceptibility to *N*-formimidoyl thienamycin emerged among the *Enterobacteriaceae* included in this study. MICs for *Escherichia coli* and *Klebsiella* spp. were 0.125 to 1 μg/ml, whereas MICs for the other species

TABLE 2. Ratios of MIC_{90}s of β-lactamase-producing and -nonproducing gram-negative bacilli

Organism	MIC_{90}					
	Mezlocillin	Cefuroxime	Cefoperazone	Cefotaxime	Moxalactam	*N*-Formimidoyl thienamycin
Escherichia coli	1	2	64	1	4	1
Klebsiella spp.	1	4	256	1	0.5	1
Enterobacter spp.	128	32	128	64	32	1
Serratia spp.	8	1	64	1	1	1
Citrobacter spp.	16	64	32	16	2	1
Proteus mirabilis	1	0.008	8	1	1	1
Proteus vulgaris	1	1	128	0.008	0.5	1
Morganella morganii	16	2	128	8	0.5	0.5
Providencia rettgeri	4	4	256	32	8	1
Pseudomonas aeruginosa	4	NT[a]	32	32	4	1

[a] NT, Not tested.

ranged from 1 to 8 μg/ml. *Proteus mirabilis* results were typical, with an MIC of 4 to 8 μg/ml. MBCs were less than twice the MICs for all strains tested. Many strains were inhibited by concentrations of cefotaxime or moxalactam below 0.125 μg/ml, whereas mezlocillin, cefuroxime, and cefoperazone were less active than was *N*-formimidoyl thienamycin (Table 1). Certain isolates were cefotaxime resistant (MIC, >8 μg/ml), and it was of interest to examine these for possible cross-resistance. Susceptibility to *N*-formimidoyl thienamycin was found to be independent of susceptibility or resistance to cefotaxime. There was limited cross-resistance between cefotaxime and moxalactam.

MICs and MBCs for gram-negative, nonfermenting bacilli. *N*-formimidoyl thienamycin was remarkably active against *Pseudomonas aeruginosa* and was more effective against this organism than any other agent included in this study, even azlocillin (range, 4 to 1,024; 50% MIC [MIC_{50}], 8; 90% MIC [MIC_{90}], 512 μg/ml). Cefotaxime and moxalactam were less active than azlocillin. The antibacterial activity of *N*-formimidoyl thienamycin against *Acinetobacter calcoaceticus* Lwoffi and *Acinetobacter anitratus* was noteworthy. Of these isolates, 90% were inhibited by 0.25 μg or less of *N*-formimidoyl thienamycin per ml. The MIC_{90} of the other β-lactam antibiotics, including cefotaxime and moxalactam, were much higher. In all *Acinetobacter* and *Pseudomonas* isolates, the action of *N*-formimidoyl thienamycin was bactericidal at concentrations less than twice the MIC.

Influence of β-lactamase production on the activity of β-lactam antibiotics. β-Lactamase production was evaluated in cell-free supernatant fluids from all gram-negative strains. Production of β-lactamases could not be detected in either *A. calcoaceticus* or *A. anitratus*. There was a marked difference between the MICs of mezlocillin and cefoperazone for β-lactamase-producing and -nonproducing strains of *Enterobacteriaceae*. Attention must be drawn to the β-lactamase-independent intrinsic resistance of many strains (Tables 1 and 2) to mezlocillin. Differences in susceptibility were also observed in some isolates for the newer cephalosporins cefuroxime, cefotaxime, and moxalactam. The antibacterial activity of *N*-formimidoyl thienamycin was found to be independent of the presence of β-lactamase (Tables 1 and 2). Consistent with these findings, the MICs of mezlocillin, azlocillin, and cefoperazone were high for the five *Pseudomonas aeruginosa* strains that were β-lactamase producers. The MICs of cefotaxime and moxalactam were only slightly increased for these strains; the MIC of *N*-formimidoyl thienamycin remained unchanged.

MICs and MBCs for gram-positive cocci. The antibacterial activity of *N*-formimidoyl thienamycin against *Streptococcus faecalis* was comparable to those of ampicillin and mezlocillin (Table 3). The cephalosporins were much less effective; the MIC_{90} of cefazedone was 16 times higher, and that of cefoperazone was 128 times higher than that of *N*-formimidoyl thienamycin. All β-lactam compounds tested against *Streptococcus faecalis* showed bactericidal action at concentrations twice the MIC (Table 3).

Against *Staphylococcus aureus* (oxacillin resistant), the MIC_{90} of *N*-formimidoyl thienamycin was 128- to 512-fold lower than that of oxacillin, cephalothin, or cefazedone (Table 3). There was a marked difference between the MIC and MBC of *N*-formimidoyl thienamycin (MBC_{90}/MIC_{90} ratio, 128); the MBC/MIC ratio for the other antibiotics was between 4 and 8 (Table 3).

DISCUSSION

The remarkably broad spectrum of antibacterial activity of *N*-formimidoyl thienamycin

TABLE 3. In vitro activity of *N*-formimidoyl thienamycin against *Streptococcus faecalis* and oxacillin-resistant staphylococci[a]

Compound	MIC (μg/ml)						MBC (μg/ml)					
	Streptococcus faecalis			*Staphylococcus aureus*			*Streptococcus faecalis*			*Staphylococcus aureus*		
	Range	50%	90%	Range	50%	90%	Range	50%	90%	Range	50%	90%
Oxacillin	NT[b]			4–256	32	128	NT			16–512	128	512
Ampicillin	1–4	2	2	NT			1–4	2	4	NT		
Mezlocillin	0.5–32	2	2	NT			1–32	2	4	NT		
Cephalothin	NT			2–16	8	16	NT			16–128	64	128
Cefazedone	4–64	16	32	8–64	16	64	8–128	32	32	128–256	128	256
Cefoperazone	16–256	32	128	NT			16–256	64	128	NT		
N-Formimidoyl thienamycin	0.5–8	1	1	0.063–0.25	0.125	0.25	1–16	2	2	1–32	8	32

[a] A total of 50 *Streptococcus faecalis* strains and 7 staphylococci were tested.
[b] NT, Not tested.

against both gram-negative and gram-positive bacteria has been pointed out by several investigators (1–3, 5) and is further confirmed in the present study. Our data also show that there was no difference between the new cephalosporins and *N*-formimidoyl thienamycin in cefotaxime-resistant strains of *Enterobacteriaceae*. The antibacterial activities of both cefotaxime and moxalactam against *Pseudomonas aeruginosa* were marginal, and both antibiotics entirely lacked activity against *Streptococcus faecalis*; *N*-formimidoyl thienamycin, however, had good activity against both species and, further, was bactericidal.

These observations do not agree with the findings of Romagnoli et al. (9), who observed marked differences between the MICs and MBCs of *N*-formimidoyl thienamycin for both gram-negative and gram-positive bacilli. These authors performed their studies with thienamycin, not the stabilized *N*-formimidoyl derivative, which may have been a contributing factor to this discrepancy (2, 3). Instability of the antibiotic may have contributed to their results.

N-Formimidoyl thienamycin was the only antibiotic among those tested with antibacterial activity independent of the production of β-lactamase by the gram-negative bacteria. This observation is compatible with a previous report that *N*-formimidoyl thienamycin is a poor substrate for β-lactamases (types IIIa and IVc included) (8). One may therefore assume that the barely detectable rate of hydrolysis will not be relevant for development of resistance (10).

We have observed a discrepant ratio between the MICs and MBCs only for the oxacillin-resistant *Staphylococcus aureus* isolates. The MICs of oxacillin, cephalothin, and cefazedone were much higher than that of *N*-formimidoyl thienamycin. However, the MBC_{90} of *N*-formimidoyl thienamycin exceeded the MIC_{90} by more than 32-fold. High MBCs and low MICs are characteristic of penicillins and cephalosporins for tolerant strains (6); the discrepant high ratio for *N*-formimidoyl thienamycin may be due to intrinsic features.

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