In Vitro Activity of N-Formimidoyl Thienamycin (MK0787), a Crystalline Derivative of Thienamycin

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N-Formimidoyl thienamycin (MK0787) is a derivative of thienamycin, a unique, new β-lactam antibiotic. Its activity against 285 aerobic and facultatively anaerobic clinical isolates was compared with the activities of cephalothin, ampicillin, penicillin G, ticarcillin, and tobramycin. All of the 285 isolates, with the exception of 1 Staphylococcus epidermidis isolate, were inhibited by a concentration of N-formimidoyl thienamycin of ≤8 µg/ml. More than 50% of all isolates were inhibited by the lowest concentration of N-formimidoyl thienamycin tested (0.125 µg/ml); 98% of Staphylococcus aureus and 80% of S. epidermidis isolates were inhibited by N-formimidoyl thienamycin at a concentration of 0.125 µg/ml. Only 2 of 45 enterococci were not inhibited by 1 µg of N-formimidoyl thienamycin per ml, and this drug was the most active agent tested against 162 gram-negative bacilli. It inhibited more than 95% of the gram-negative isolates at a concentration of ≤2 µg/ml. N-Formimidoyl thienamycin was as active or more active than tobramycin against Escherichia coli, Pseudomonas aeruginosa, and Proteus mirabilis and substantially more active than ticarcillin. All 16 isolates of Klebsiella pneumoniae were inhibited by ≤0.5 µg of N-formimidoyl thienamycin per ml. The marked in vitro activity of this drug against a wide variety of clinical isolates makes it a promising new antibiotic.

MK0787 (N-formimidoyl thienamycin) is a crystalline derivative of the new β-lactam antibiotic thienamycin. Thienamycin exhibits potent activity against a wide variety of aerobic and anaerobic bacteria (5, 6). However, thienamycin also possesses considerable chemical instability, remaining active only within a relatively narrow range of pH, temperature, and antibiotic concentration (2). N-Formimidoyl thienamycin has greater stability in both the solid state and solution (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).

This study presents in vitro susceptibility data on aerobic and facultatively anaerobic bacteria tested with N-formimidoyl thienamycin and other antibiotics in current use in the treatment of infections with common pathogens. In contrast to investigations done in academic and university institutions, which may utilize relatively resistant organisms, isolates for the present study were collected sequentially from clinical infections encountered in a large community hospital.

MATERIALS AND METHODS

Microorganisms. The microorganisms tested included 285 aerobic and facultatively anaerobic isolates randomly selected from inpatients and outpatients at the emergency room of St. Paul Hospital, Dallas, Tex. The isolates included 123 gram-positive cocci and 162 gram-negative bacilli. Of the 123 gram-positive coccus isolates, 60% were isolated from wounds; the remainder were from urine, blood, and respiratory tract specimens. Of the 162 isolates of gram-negative bacilli, 65% were urine isolates; the remainder were isolated from blood, wounds, respiratory tract specimens, and stools.

The organisms were maintained on Mueller-Hinton agar plates and periodically subcultured to fresh plates until the time of susceptibility testing. Identification of the gram-positive isolates was in accord with the criteria of Blair et al. (1). Gram-negative isolates were identified primarily with the API 20E System (Analytab Products, Inc., Plainview, N.Y.), and these identifications were confirmed by the criteria of Blair et al.

Antibiotics. Standard antibiotic powders were obtained from the following sources: N-formimidoyl thienamycin from Merck Institute for Therapeutic Research, Rahway, N.J.; tobramycin and cephalothin from Eli Lilly & Co., Indianapolis, Ind.; penicillin G from Bristol Laboratories, Syracuse, N.Y.; ampicillin from Wyeth Laboratories, Philadelphia, Pa.; and ti-
carcinillin from Beecham Laboratories, Bristol, Tenn. All antibiotic solutions were prepared on the day used. N-Formimidoyl thienamycin was prepared in 10 mM potassium phosphate buffer, pH 7.0. The concentration of N-formimidoyl thienamycin was confirmed by measuring the absorbance of the drug at 299 nm with an Aminco model DW2 spectrophotometer (American Instruments, Bethesda, Md.). The measured concentration of N-formimidoyl thienamycin was within 2% of the calculated level of the drug. The following concentrations of antibiotics (in micrograms per milliliter) were tested: N-formimidoyl thienamycin, 0.125 to 32; cephalothin, 0.5 to 16; ampicillin, 0.125 to 16; penicillin G, 0.0625 to 8; ticarcillin, 4 to 256; and tobramycin, 0.25 to 16. Those gram-positive cocci which were inhibited by 0.5 µg of cephalothin per ml and those gram-negative bacilli which were inhibited by 4 µg of ticarcillin per ml were tested subsequently with further twofold dilutions to a concentration of 0.125 µg/ml. Dilutions of the antibiotics were performed with Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.), which was supplemented for the determination of tobramycin susceptibility with CaCl₂ and MgCl₂ to contain 5 mg of calcium per dl and 2.5 mg of magnesium per dl, respectively.

Determination of minimal inhibitory concentrations. Bacterial inocula were prepared by diluting a late logarithmic-phase culture in Mueller-Hinton broth to a McFarland number 1 standard and then further diluting with sterile normal saline to a concentration of approximately 4 X 10⁷ colony-forming units per ml. The diluted bacterial inoculum (1 µl) was added to 100 µl of an antibiotic solution in each well of a Dynatech MIC-2000 (Dynatech Laboratories, Alexandria, Va.) dispensing instrument (3). Inoculated plates were incubated at 37°C in a room air incubator. The plates were read with the aid of a magnifying mirror after 15 to 18 h of incubation. The lowest concentration of an antibiotic which showed no visible turbidity or deposit at the bottom of the microtiter well was considered the minimal inhibitory concentration of that antibiotic. Minimum bactericidal concentrations were not determined. Staphylococcus aureus ATCC 29213, Streptococcus faecalis ATCC 29212, Pseudomonas aeruginosa ATCC 27853, and Escherichia coli ATCC 25922 were used as control strains.

Determination of β-lactamase activity. β-Lactamase activities of staphylococcal isolates were tested with the chromogenic cephalosporin nitrocefin (compound 87/312) (Glaxo Research Ltd., Greenford, England), using the method described by Montgomery et al. (4).

RESULTS

The comparative in vitro activities of N-formimidoyl thienamycin, penicillin G, ampicillin, cephalothin, tobramycin, and ticarcillin are shown in Table 1.

N-Formimidoyl thienamycin appeared to be the most active agent against S. aureus, Staphylococcus epidermidis, and enterococci. More than 90% of all staphylococci were inhibited by N-formimidoyl thienamycin at a concentration of 0.125 µg/ml. Fourfold-greater concentrations of cephalothin were required to inhibit 90% of the S. aureus isolates; against S. epidermidis, eightfold-greater concentrations were required (Fig. 1).

N-Formimidoyl thienamycin inhibited both β-lactamase-producing and non-β-lactamase-producing staphylococcal isolates. All 51 β-lactamase-producing isolates of S. aureus and 6 of 7 (86%) β-lactamase-producing isolates of S. epidermidis were inhibited by 0.125 µg of N-formimidoyl thienamycin per ml.

Only 2 of 45 enterococci were not inhibited by 1 µg of N-formimidoyl thienamycin per ml. Only one isolates was inhibited by that concentration of ampicillin (Fig. 2). All nine isolates of group B streptococci were inhibited by the lowest concentrations of N-formimidoyl thienamycin, cephalothin, and penicillin G tested.
The activity of N-formimidoyl thienamycin against gram-negative bacilli compared favorably with the activities of tobramycin and ticarcillin. N-Formimidoyl thienamycin inhibited more than 95% of the 162 gram-negative isolates when tested at a concentration of 2 μg/ml. At this same concentration, ticarcillin and tobramycin inhibited 25 and 80% of the isolates, respectively. As Fig. 3 shows, N-formimidoyl thienamycin was as active or more active than tobramycin against E. coli and P. aeruginosa and substantially more active than ticarcillin against these organisms. Against 23 isolates of Proteus mirabilis, the activities of all of these drugs were similar. Although only a few isolates were available for testing, N-formimidoyl thienamycin displayed greater activity than either ticarcillin or tobramycin against indole-positive Proteus species.

More than 85% of the 16 Klebsiella pneumoniae isolates were inhibited by ≤0.25 μg of N-formimidoyl thienamycin per ml, whereas a concentration of 1 μg of tobramycin per ml was required to inhibit the same percentage of these isolates. Both drugs were substantially more active than ticarcillin. Similar differences in activ-
ity among these three antimicrobial agents were observed with five isolates of Serratia marcescens. A total of 12 isolates of Enterobacter were tested, including 7 E. aerogenes isolates, 4 E. cloacae isolates, and 1 E. agglomerans isolate. There were only minor variations in the minimal inhibitory concentrations of these same antibiotics among the different species. All organisms were inhibited by \( \leq 2 \mu g \) of N-formimidoyl thienamycin per ml. The cumulative distribution curves are shown in Fig. 4.

A total of 14 additional gram-negative bacillary isolates were tested, including Citrobacter spp. (five isolates), Klebsiella oxytoca (two isolates), Acinetobacter calcoaceticus subsp. anitratus (two isolates), Providencia stuartii (two isolates), Shigella sonnei (one isolate), Aeromonas hydrophila (one isolate), and Salmonella sp. (one isolate). All were inhibited by \( \leq 2 \mu g \) of N-formimidoyl thienamycin per ml. Ticarcillin and tobramycin inhibited 20 and 85%, respectively, of these organisms at the same concentration.

More than one-half of the 285 isolates tested were inhibited by 0.125 \( \mu g \) of N-formimidoyl thienamycin per ml, the lowest concentration used for our in vitro tests, and 98% were inhibited by \( \leq 2 \mu g/ml \). All of the isolates tested, with the exception of one S. epidermidis isolate, were inhibited by \( \leq 8 \mu g \) of N-formimidoyl thienamycin per ml; this single S. epidermidis isolate was not inhibited by 32 \( \mu g/ml \).

**DISCUSSION**

This study demonstrates that N-formimidoyl thienamycin is a highly active antimicrobial agent which is capable of inhibiting in vitro a variety of aerobic and facultatively anaerobic bacteria. This drug is a crystalline derivative of thienamycin, a bactericidal cell wall-active antibiotic (5). N-Formimidoyl thienamycin has en-

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**Fig. 3.** Cumulative distribution curves of susceptibilities of isolates of E. coli, P. aeruginosa, and P. mirabilis to N-formimidoyl thienamycin (MK0787), ticarcillin, and tobramycin.

**Fig. 4.** Cumulative distribution curves of susceptibilities of isolates of K. pneumoniae, Enterobacter spp., and S. marcescens to N-formimidoyl thienamycin (MK0787), ticarcillin, and tobramycin.
hanced chemical stability and, in general, enhanced potency compared with its parent compound.


In the present study, 284 of the 285 unselected gram-positive cocci and gram-negative bacilli isolated from clinical specimens in a community hospital were inhibited by a concentration of ≤8 μg of N-formimidoyl thienamycin per ml. We did not evaluate its activity against obligate anaerobic organisms, but Kropp et al. found substantial in vitro activity of N-formimidoyl thienamycin against Bacteroides fragilis (H. Kropp, J. G. Sundelof, J. S. Kahan, F. M. Kahan, and J. Birnbaum, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 19th, Boston, Mass., abstr. no. 231, 1979).

Because of its remarkable activity against many clinically encountered human bacterial pathogens, this agent appears to be especially promising. Further studies of its pharmacokinetics, in vivo activity, and toxicity will be of interest.

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LITERATURE CITED


