

N-Formimidoyl Thienamycin (MK0787): In Vitro Activity Against Anaerobic Bacteria

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Received 27 April 1981/Accepted 28 September 1981

The in vitro activity of *N*-formimidoyl thienamycin (MK0787) was tested against 239 anaerobic bacteria clinical isolates: 70 of *Bacteroides fragilis*, 18 of *B. distasonis*, 16 of *B. thetaiotaomicron*, 10 of *B. vulgatus*, 24 of *Bacteroides* spp., 22 of *B. melaninogenicus* (all three subspecies), 26 of *Fusobacterium* spp., 10 of *Peptococcus* spp., 15 of *Peptostreptococcus* spp., 15 of *Clostridium perfringens*, and 13 of *Clostridium* spp. Ninety-five percent of the isolates were inhibited by ≤ 0.125 $\mu\text{g/ml}$, and all were inhibited by ≤ 4 $\mu\text{g/ml}$.

The increasing awareness that anaerobic bacteria may cause serious infections has spurred a search for agents that exhibit greater activities against anaerobes. Thienamycin, a beta-lactam antibiotic produced by the soil organism *Streptomyces cattleya*, has been shown to have activity against many aerobic and selected anaerobic (including *Bacteroides fragilis*) bacteria (9-11). *N*-Formimidoyl thienamycin (MK0787), obtained by chemical modification of thienamycin, is more stable than the parent antibiotic both in its crystalline solid state and in concentrated solutions (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). Previous limited studies have documented the in vitro activity of *N*-formimidoyl thienamycin against anaerobic bacteria (4, 5, 9, 10). We investigated the in vitro activity of this antibiotic against a variety of anaerobes recovered from clinical specimens.

A total of 239 anaerobic bacteria were tested. Most isolates were recovered from clinical specimens obtained from patients hospitalized at Charity Hospital of Louisiana at New Orleans and submitted to the Louisiana State University Infectious Disease Laboratory. These isolates had been maintained frozen in skim milk at -70°C . Before use they were subcultured on reduced 5% sheep blood agar plates and incubated at 35°C for 48 h in an anaerobic chamber (National Appliance Co., Hollywood, Fla.) containing an anaerobic gas mixture of 85% nitrogen, 10% hydrogen, and 5% carbon dioxide. Gram-positive cocci and *Fusobacterium* species were allowed to grow for 72 h to obtain larger isolated colonies. The anaerobic bacteria had been isolated and characterized as previously described (3). *B. fragilis* ATCC 25285, *B. thetaiotaomicron* ATCC 29741, and *Clostridium*

perfringens ATCC 13124 were obtained from the American Type Culture Collection, Rockville, Md. As previously recommended, one or more of these control strains were included in each group of anaerobes tested (8).

All frozen isolates were subcultured onto 5% sheep blood agar plates supplemented with hemin (5 $\mu\text{g/ml}$) and menadione (0.5 $\mu\text{g/ml}$) that had been reduced overnight in GasPak jars (BBL Microbiology Systems, Cockeysville, Md.). Wilkins-Chalgren broth was prepared as previously described, excluding the 1.5% (wt/vol) agar (12). Wilkins-Chalgren broth was used in plate preparation and for subculturing of the *B. fragilis* group, the *Bacteroides* species, and the *Clostridium* species. For *Peptococcus* species, *Peptostreptococcus* species, *Fusobacterium* species, and *B. melaninogenicus*, heat-inactivated (56°C for 0.5 h) horse serum was added to Schaedler broth (BBL) to a final concentration of 1% before use in subculturing and plate preparation (2).

N-Formimidoyl thienamycin was supplied by Merck & Co., Inc., Rahway, N.J. Minimum inhibitory concentrations (MICs) were determined using the method previously described by Fass (2), with the inoculum preparation modified according to the procedure described by MicroMedia Systems for use with their anaerobic panels (*Microdilution Anaerobe MIC Panels, Procedure for Use*, p. 2). Based on colony counts, the colony-forming units of the inoculum ranged from 1×10^5 to 5×10^6 per ml.

N-Formimidoyl thienamycin showed marked activity against the anaerobic bacteria tested (Table 1). All isolates were inhibited by ≤ 4 $\mu\text{g/ml}$, and 95% were inhibited by ≤ 0.125 $\mu\text{g/ml}$. The only isolates that required more than 0.5 $\mu\text{g/ml}$ were a few strains of *B. fragilis* and *Clostridium* species.

We found that *N*-formimidoyl thienamycin

TABLE 1. In vitro susceptibility testing of *N*-formimidoyl thienamycin

Organism	No. of strains	MIC ($\mu\text{g/ml}$)		
		Range	50%	90%
<i>B. fragilis</i>	70	≤ 0.004 –4	0.062	0.25
<i>B. thetaiotaomicron</i>	16	0.008–0.125	0.031	0.125
<i>B. distasonis</i>	18	0.008–0.25	0.062	0.125
<i>B. vulgatus</i>	10	0.016–0.125	0.062	0.125
<i>Bacteroides</i> spp.	24	≤ 0.004 –0.125	0.016	0.062
<i>B. melaninogenicus</i> ^a	22	≤ 0.004 –0.125	0.016	0.062
<i>Fusobacterium</i> spp.	26	≤ 0.004 –0.5	0.016	0.5
<i>Peptococcus</i> spp.	10	≤ 0.004 –0.125	0.031	0.125
<i>Peptostreptococcus</i> spp.	15	≤ 0.004 –0.062	≤ 0.004	0.031
<i>C. perfringens</i>	15	0.008–4	0.062	4
<i>Clostridium</i> spp.	13	≤ 0.004 –1	0.062	0.25

^a Includes all three subspecies.

was extremely active against all of the anaerobes tested. Kropp and co-workers (5) tested 29 isolates of *B. fragilis* by agar dilution and found that 90% were inhibited by 1 $\mu\text{g/ml}$. Brown et al. (1) tested 100 isolates of *B. fragilis* by agar dilution and found that 50% were inhibited by ≤ 0.06 $\mu\text{g/ml}$, and 90% were inhibited by 0.25 $\mu\text{g/ml}$. Kesado and co-workers (4) reported MICs ranging from 0.031 to 4 $\mu\text{g/ml}$ for five anaerobic reference strains. Tally and co-workers (10) reported that 84 species of anaerobic bacteria were inhibited by ≤ 2 $\mu\text{g/ml}$ (56 *B. fragilis* isolates, 14 anaerobic gram-positive cocci, and 14 *Clostridium* species). Our MIC values agree with those reported by the investigators mentioned above. In testing a greater number of isolates, we found that 100% were inhibited by ≤ 4 $\mu\text{g/ml}$.

New antimicrobial agents with activity against anaerobes are needed because of the increasing resistance of anaerobic bacteria (including *B. fragilis*) to the drugs currently in use and the toxicity of these approved drugs (6, 7). *N*-Formimidoyl thienamycin, because of its broad anaerobic spectrum, may be potentially useful in the treatment of multiple types of anaerobic infections. The clinical efficacy of this antibiotic will have to be substantiated in further trials.

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