In Vitro Activity of Mecillinam Against Anaerobic Bacteria

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A microtiter broth dilution method was employed to determine the in vitro activity of mecillinam against 201 recent clinical isolates of anaerobic bacteria. Both the anaerobic gram-positive and anaerobic gram-negative bacilli displayed a wide range of minimal inhibitory concentrations of mecillinam; most strains were resistant to the antibiotic. The anaerobic cocci exhibited a narrower range of minimal inhibitory concentrations than were observed with other anaerobes, but also exhibited mecillinam resistance. As a single drug, mecillinam does not appear to be an effective antimicrobial agent against anaerobic bacteria.

Mecillinam, a new beta-lactam antibiotic, was first described by Lund and Tybring (4). This antibiotic belongs to a new and novel class of penicillins, the amidino penicillins. In general, penicillins, whether natural or semisynthetic, are acyl derivatives of 6-aminopenicillanic acid. Mecillinam is a 6 beta-amidopenicillanic acid derivative (4). In contrast to most penicillins, generally are more active against gram-positive organisms, mecillinam possesses a remarkably high activity against many members of the Enterobacteriaceae. Mecillinam especially is active against Escherichia coli, Klebsiella pneumoniae, Enterobacter species, Citrobacter species, Salmonella, and Shigella, but is not active against Pseudomonas aeruginosa (5, 11). Mecillinam is relatively inactive against gram-positive bacteria such as staphylococci and streptococci (5, 6, 13). The activity of mecillinam against anaerobic bacteria has been tested on only a limited basis (5, 10). The purpose of this study was to further investigate the activity of mecillinam against a wide range of anaerobic bacteria.

MATERIALS AND METHODS

All anaerobic bacteria employed in this study were recent clinical isolates recovered from wound, lower respiratory, and genital tract specimens submitted to the Clinical Microbiology Laboratories of the North Carolina Memorial Hospital, Chapel Hill. All anaerobic bacteria employed for testing were first identified through the use of gas-liquid chromatography and specialized anaerobic biochemical tests (5). The following anaerobic bacteria were selected for study: 6 strains of Bacteroides fragilis, 15 B. melaninogenicus subsp. intermedium, 29 B. fragilis, 11 B. vulgatus, 15 B. distasonis, 7 B. ovatus, 22 B. thetaiotaomicron, 6 Clostridium tertium, 18 C. perfringens, 5 C. bifermentans, 5 C. innocuum, 9 Propionibacterium acnes, 6 Propionibacterium granulosum, 6 Peptococcus prevoti, 5 Peptococcus asaccharolyticus, 15 Peptostreptococcus anaerobius, 6 Peptostreptococcus micros, 5 Eubacterium lentum, and 10 Veillonella parvula.

The in vitro activity of mecillinam against anaerobic bacteria was investigated by employing a microbroth dilution method (8). By testing reference strains of B. fragilis ATCC 25285 and C. perfringens ATCC 13124 against penicillin G, cefoxitin, and clindamycin, this method was shown to yield results similar to those obtained with the reference agar dilution method (9). Mecillinam powder was supplied by Hoffmann-La Roche, Inc., Nutley, N.J. The other antibiotic powders were supplied as follows: penicillin G, Eli Lilly and Co., Indianapolis, Ind.; cefoxitin, Merck Sharp and Dohme, Inc., West Point, Pa.; and clindamycin Peptococcus fragilis, Upjohn Co., Kalamazoo, Mich. The mecillinam powder initially was dissolved in sterile distilled water to yield a concentration of 1,280 µg/ml. Twelve serial twofold dilutions ranging between 128 and 0.06 µg/ml were then prepared in sterile NIH broth supplemented with 5 µg of hemin and 0.1 µg of menadione per ml. NIH broth was prepared by dissolving 5 g of yeast extract (Difco Laboratories, Detroit, Mich.), 15 g of trypticase-peptone (BBL Microbiology Systems, Cockeysville, Md.), 1.0 g of dextrose, 2.5 g of sodium chloride, and 0.05 g of L-cysteine in 1 liter of distilled water. The medium was sterilized by autoclaving for 15 min at 121°C. The sterilized medium was then supplemented with hemin and menadione to yield final concentrations of 5 and 0.1 µg/ml, respectively. The osmolarity of the supplemented NIH broth was determined to be 210 msmol/kg through use of an Advanced Digimatic Osmeter, model 3D (Advanced Instruments Co., Inc., Needham Heights, Mass.).

Portions (50 µl) of the mecillinam dilutions were aseptically dispensed into sterile U-bottom microtiter trays (Cooke Laboratories, Inc., Alexandra, Va.) with a Dynatech MIC-2000 dispenser (Cooke Laboratories, Inc.). Prepared microtiter trays not intended for use within 24 h of preparation were sealed in polyethylene bags and frozen at −70°C for a maximum of 2 weeks. Microtiter plates were prepared for inoculation by first incubating them at room temperature for 24 h in an
anaerobic glove box (Coy Laboratory Products, Ann Arbor, Mich.) containing an atmosphere of 85% nitrogen, 10% hydrogen, and 5% carbon dioxide.

The stability of mecillinam in the prepared microtiter trays was monitored by testing a strain of *B. fragilis* (for which the minimal inhibitory concentration [MIC] of mecillinam was known) on each day tests were performed. This quality control testing indicated that the antibiotic was stable for at least 2 weeks under the storage, prereduction, and incubation conditions employed. It also indicated that the antibiotic was not inactivated by the L-cysteine in the test medium.

Organisms tested were first grown in the glove box environment at 35°C for 24 to 48 h on an agar medium which consisted of the previously described NIH broth supplemented with 1.5% agar and 5% defibrinated sheep blood (Brown Laboratories, Topeka, Kans.). Inocula were prepared by suspending well-isolated colonies of each test strain from NIH blood agar plates in 2 ml of supplemented Schaedler broth. The turbidity of the organism suspension was adjusted to equal that of a McFarland no. 0.5 turbidity standard. This standardized suspension then was diluted 1:100 in supplemented NIH broth. Volumes (50 μl) of the diluted suspension were added to each antibiotic-containing well and to a growth control well containing 50 μl of supplemented broth without antibiotics. With inoculation, the prepared antibiotic concentrations were further diluted to yield concentrations of mecillinam that ranged between 64 and 0.03 μg/ml. Incubated plates were sealed in polyethylene bags and incubated in a glove box at 35°C for 48 h. The incubated plates were examined with an illuminated viewer (Cooke Laboratories, Inc.), and the MIC was determined as the lowest concentration of antibiotic that inhibited visible growth.

**RESULTS**

Table 1 presents the results obtained for the 201 strains of anaerobic bacteria tested. *C. bifermentans* and *E. lentum* were the most susceptible of the anaerobic gram-positive bacilli tested. MICs for *C. bifermentans* ranged between 0.25 and 4.0 μg/ml, and MICs for *E. lentum* ranged between 1.0 and 4.0 μg/ml. More elevated MICs were observed for *P. acnes* and *P. granulosum*: between 2.0 and 16.0 μg/ml and between 0.5 and 16 μg/ml, respectively. *C. tertium*, *C. innocuum*, and *P. perfringens* were the least susceptible of the anaerobic gram-positive bacilli. MICs for *P. perfringens* ranged between 8.0 and 64 μg/ml, whereas *C. innocuum* displayed MICs that ranged between 16 and 64 μg/ml. *C. tertium* demonstrated MICs that ranged between 8 and 32 μg/ml.

In comparison to the anaerobic gram-positive bacilli, the anaerobic gram-negative bacilli displayed a wider range of MICs. *B. corrodens* and *B. vulgatus* displayed MICs that ranged between 0.125 and 16.0 μg/ml. MICs for *B. melaninogenicus* subsp. *intermedius* ranged between 0.125 and 16 μg/ml, and strains of *B. distasonis* displayed MICs that ranged between ≤0.06 and

| Organism                        | No. of strains | MIC (μg/ml)       |
|--------------------------------|----------------|-------------------|
|                                |                | Range            | For 50% of strains | For 90% of strains |
| Anaerobic gram-positive bacilli|                |                  |                   |
| *Clostridium tertium*           | 6              | 8-32             | 16                | 32               |
| *C. bifermentans*               | 5              | 0.25-4           | 2                 | 4                |
| *C. innocuum*                  | 5              | 16-64            | 16                | 64               |
| *C. perfringens*               | 18             | 8-64             | 16                | 64               |
| *Propionibacterium acnes*      | 9              | 2-16             | 4                 | 8                |
| *P. granulosum*                | 6              | 0.5-16           | 2                 | 4                |
| *Eubacterium lentum*           | 5              | 1-4              | 2                 | 4                |
| Anaerobic gram-negative bacilli|                |                  |                   |
| *Bacteroides corrodens*         | 6              | 0.125-16         | 0.5               | 16               |
| *B. melaninogenicus* subsp. *intermedius* | 15 | 0.25-16 | 2 | 16 |
| *B. fragilis*                  | 29             | 1-64             | 8                 | 32               |
| *B. distasonis*                | 15             | 0.06-16          | 1                 | 16               |
| *B. thetaiotaomicron*           | 22             | 4-64             | 8                 | 32               |
| *B. vulgatus*                  | 11             | 0.125-16         | 0.5               | 8                |
| *B. ovatus*                    | 7              | 2-32             | 4                 | 16               |
| Anaerobic cocci                |                |                  |                   |
| *Peptostreptococcus anaerobius* | 15             | 0.125-16         | 2                 | 8                |
| *P. micros*                    | 6              | 0.5-8            | 2                 | 4                |
| *Peptococcus asaccharolyticus*  | 5              | 0.5-8            | 2                 | 4                |
| *P. prevotii*                  | 6              | 1-8              | 2                 | 4                |
| *Veillonella parvula*           | 10             | 0.5-4            | 1                 | 2                |
16 μg/ml. *B. thetaiotaomicron* and *B. fragilis* were the most resistant of the anaerobic gram-negative bacilli; each required 64 μg/ml for inhibition of all strains. A mecinillin concentration of 32 μg/ml was required for the inhibition of all seven strains of *B. ovatus* tested.

*V. parvula* was the only anaerobic gram-negative coccus tested and displayed MICs of approximately 0.5 and 4.0 μg/ml. Of the anaerobic gram-positive cocci, all strains of *P. micros*, *P. prevotii*, and *P. asaccharolyticus* demonstrated MICs of ≤8.0 μg of mecinillin per ml. *P. anaerobius* displayed a wider range of MICs, with values ranging between 0.125 and 16 μg/ml. Approximately 93% of the *P. anaerobius* strains were susceptible to 8.0 μg/ml, whereas approximately 7% required 16 μg/ml for inhibition.

**DISCUSSION**

Since mecinillin is poorly absorbed from the gastrointestinal tract, effective oral therapy originally was not possible, and the antibiotic was administered parenterally (7). Recently, an effective oral preparation has been developed. Pivmecillinam, the pivaloyloxymethyl ester of mecinillin, possesses no antibacterial activity per se but is well absorbed from the gastrointestinal tract. During or immediately after absorption, pivmecillinam is enzymatically hydrolyzed to mecinillin (7).

Roholt and co-workers, in a study of 9 patients, found peak serum levels after intramuscular administration of either 250 or 500 mg of mecinillin to be approximately 8 and 10 μg/ml, respectively (7). The serum half-life of mecinillin intramuscularly administered was approximately 1 h (7). In a study of 10 fasting volunteers, Roholt and co-workers found mean peak serum levels after the oral administration of 100 mg of pivmecillinam to be approximately 5 μg/ml, with a serum half-life of approximately 1 h (7).

Assuming a susceptible breakpoint for mecinillin to equal 4.0 μg/ml, this antibiotic appears relatively inactive against anaerobic bacteria. *B. fragilis* and *C. perfringens*, two of the most commonly isolated anaerobic bacteria, are highly resistant to mecinillin; none of the strains of *C. perfringens* tested, and only about 34% of the strains of *B. fragilis*, were susceptible to ≤4.0 μg of mecinillin per ml. *C. bifermrmentans*, *E. lentum*, and *V. parvula* were the only anaerobic bacteria that demonstrated consistent susceptibility to mecinillin. Resistance among other anaerobic bacteria was frequently encountered. Of all the anaerobic strains tested, for only 52.7% was the MIC ≤4.0 μg/ml. It is, therefore, extremely difficult to predict susceptibility to mecinillin. Since the susceptibility of the majority of anaerobic bacteria tested cannot be predicted, susceptibility testing is mandatory if mecinillin is ever to be considered for treatment of anaerobic infections.

In vitro susceptibility testing of mecinillin requires a medium with the proper osmolality and conductivity. Susceptibility testing media with high osmolality and conductivity have been demonstrated to significantly reduce the activity of mecinillin (1, 2, 12). The osmolality and conductivity of the medium we employed were sufficiently low to insure accurate measurements of antibiotic activity.

Although mecinillin is active against many aerobic gram-negative bacilli, its potential use for the treatment of anaerobic infections appears limited. Should consideration be given to the clinical use of mecinillin for the treatment of anaerobic infections, in vitro susceptibility testing is strongly indicated.

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