In Vitro Susceptibility of *Nocardia asteroides* to *N*-Formimidoyl Thienamycin and Several Cephalosporins

MICHAEL H. CYNAMON†‡ and GREGORY S. PALMER

Department of Medicine, Veterans Administration Medical Center and State University of New York, Upstate Medical Center, Syracuse, New York 13210

Received 4 August 1981/Accepted 11 September 1981

The susceptibility of *N. asteroides* to *N*-formimidoyl thienamycin, cefamandole, cefoxitin, and moxalactam was determined by agar dilution. *N*-Formimidoyl thienamycin was the most active, inhibiting eight of nine strains at 1.56 μg/ml.

In the United States, nocardial infections are caused predominantly by *Nocardia asteroides*. Pulmonary nocardiosis is the most common type of nocardial infection. Hematogenous dissemination frequently occurs, involving the central nervous system, soft tissues, or both. Infection can occur in patients who are not immunocompromised; however, the factors which are most commonly associated with infection include corticosteroid therapy, immunosuppressive therapy, hematological malignancy, bronchopulmonary abnormalities, and pulmonary alveolar proteinosis.

Sulfonamides remain the agents of choice (5). In a significant number of patients sulfonamides are not well tolerated. Other antibacterial agents such as minocycline (1), erythromycin (6), ampicillin (2), and amikacin (3, 7) have been proposed as potential alternative agents based on in vitro susceptibility studies. Our recent in vitro studies with several of the newer cephalosporins against *Mycobacterium fortuitum* prompted us to study their in vitro activities against *N. asteroides*, a closely related member of the Actinomycetales order.

Isolates of *N. asteroides* were provided by Morris Gordon, Division of Laboratories and Research, New York State Department of Health, Albany, N.Y., and several were clinical isolates from the Veterans Administration Medical Center, Syracuse, N.Y. *Staphylococcus aureus* ATCC 25923 (Difco Laboratories, Detroit, Mich.) and *Escherichia coli* ATCC 29194 (Difco) were used as control organisms.

The antimicrobial agents evaluated in this study were provided as standard powders as follows: cefoxitin (potency, 946 μg/mg) and *N*-formimidoyl thienamycin (potency, 1,000 μg/mg) from Merck Institute for Research, Rahway, N.J.; and cefamandole (potency, 888 μg/mg) and moxalactam (potency, 1,000 μg/mg) from Eli Lilly & Co., Indianapolis, Ind. We prepared stock solutions of each antimicrobial agent immediately before use by hydrating a known weight of each drug in distilled water (10 mM phosphate buffer [pH 7.2] for *N*-formimidoyl thienamycin) and then sterilizing the drug by filtration through a GA-6 0.45 μm membrane filter (Gelman Sciences, Inc., Ann Arbor, Mich.).

*Nocardia* grown on Mueller-Hinton agar (Difco) were inoculated into tubes containing 10 ml of Mueller-Hinton broth (Difco) with 0.05% Tween 80 (MHB-T80) and five 4-mm glass beads (Arthur Thomas Co., Philadelphia, Pa.) to decrease aggregation of the nocardial cells. The cultures were grown for 48 h in a shaking water bath at 37°C. They were then diluted with MHB-T80 to 1 and 0.1 Klett unit/ml (Klett-Summerson Colorimeter; Klett Manufacturing, Brooklyn, N.Y.). One Klett unit yielded approximately 10^6 colony-forming units (range, 7.6 × 10^5 to 2.4 × 10^6 colony-forming units). The *E. coli* and *S. aureus* were grown overnight and diluted as described above; they yielded 2.5 × 10^7 and 2.3 × 10^6 colony-forming units per Klett unit, respectively.

Agar dilution susceptibility tests were performed in quadrant plates with serial twofold dilutions of cefoxitin, cefamandole, moxalactam (range, 50 to 0.39 mg/ml), and *N*-formimidoyl thienamycin (range, 25 to 0.19 mg/ml) in Mueller-Hinton agar. The plates were spotted in duplicate with 10 μl of culture. They were then incubated at 37°C for 72 h, and the minimum inhibitory concentration was defined as the lowest antibiotic concentration which yielded no perceptible growth. The *E. coli* and *S. aureus* plates were read after 24 h.

The aga dilution minimum inhibitory concentrations of the *β*-lactam antibiotics against *N. asteroides*, determined for the 0.1-Klett unit/ml cultures, are listed in Table 1. The minimum inhibitory concentrations for the 1-Klett unit/ml cultures.
ml cultures were similar except for being occasionally one dilution higher than those for the 0.1-Klett unit/ml cultures. Cefamandole inhibited all of the strains of nocardia tested at 25 μg/ml and five of nine strains at 12.5 μg/ml. Cefoxitin inhibited seven of nine strains at 50 μg/ml. Only one strain, H80, was susceptible below this level. Moxalactam inhibited eight of nine strains at 25 μg/ml and four of nine strains at 12.5 μg/ml. N-Formimidoyl thienamycin inhibited all of the strains tested at 6.25 μg/ml and eight of nine strains at 1.56 μg/ml.

There are few reports of the in vitro activity of cephalosporins against Nocardia spp. Lerner and Baum (6) found cephaloridine to be more active than cephalothin or cephalaxin, inhibiting approximately 65% of the isolates tested at 12.5 μg/ml and 80% at 50 μg/ml. Ampicillin inhibits almost 50% of the isolates at 12.5 μg/ml. Oxacillin, dicloxacinil, nafcillin, and penicillin G are much less active. Bach et al. (1) found cephaloridine to be the most active of the cephalosporins they tested. It inhibits 59% of the strains at 50 μg/ml. Cefoxitin inhibits 50% of the strains at 50 μg/ml. Cephapirin, cefazolin, and cephalone are somewhat less active than cephaloridine. More recently, Garcia-Rodriguez et al. (4) reported on the in vitro activity of cefoxitin against Nocardia and Actinomadura spp. Cefoxitin inhibits 91% of the N. asteroides isolates tested at 12.5 μg/ml. The variation in the activity of cefoxitin may be explained by differences in the methodology used for susceptibility testing and by the population of organisms tested.

In this study, cefamandole and moxalactam were more active than cefoxitin. N-Formimidoyl thienamycin was very active, inhibiting most of the strains tested at 1.56 μg/ml. This β-lactam antibiotic appears to be a potentially useful agent for the treatment of infections caused by N. asteroides.

Nocardia have been shown to have β-lactamas; however, the importance of these β-lactamas in the resistance of these organisms to β-lactam antibiotics remains unclear (8). For 15 of 16 strains tested, Wallace et al. (8) demonstrated a fourfold or greater reduction in the minimum inhibitory concentration of ampicillin in the presence of subinhibitory concentrations of cloxacinil. There was a poor correlation between β-lactamase production in nocardia and their susceptibility to β-lactam antibiotics. Permeability, the ability of the penicillin-binding proteins to interact with the substrate, or both likely play a more important role than the presence of β-lactamas in determining the susceptibility of these organisms to the β-lactam antibiotics.

LITERATURE CITED