

## Synergism of Imipenem and Amikacin in Combination with Other Antibiotics Against *Nocardia asteroides*

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The in vitro activities of imipenem (formerly imipemide, *N*-formimidoyl thienamycin, or MK0787) and amikacin in combination with cefotaxime, trimethoprim-sulfamethoxazole, and each other were tested against 26 *Nocardia asteroides* strains. The agar dilution method was used for all tests. Synergy was present in 80% of tests with imipenem-trimethoprim-sulfamethoxazole, in 92% of tests with imipenem-cefotaxime, and in 83% of tests with amikacin-trimethoprim-sulfamethoxazole. Indifference was found on rare occasions, and no antagonism was seen.

Several antibiotics, including amikacin, cefotaxime, and imipenem (formerly imipemide, *N*-formimidoyl thienamycin, or MK0787), among others, have been shown to be efficacious against *Nocardia asteroides* (3). The antibiotic combination of trimethoprim-sulfamethoxazole (TMP-SMX) has less activity than the aforementioned agents on a weight basis in vitro but has proven efficacy in vivo and has been advocated as the agent of choice in the therapy of nocardial infections (6). There have been reports, however, of unsuccessful therapy when single agents have been used, and combination antimicrobial therapy has proven to be superior to single-agent therapy (2, 4).

In this study, the minimal inhibitory concentrations (MICs) of imipenem, amikacin, cefotaxime, and TMP-SMX for 26 *N. asteroides* strains were determined by the agar dilution technique. In addition, synergy studies were performed with the following antibiotic combinations: imipenem-TMP-SMX, imipenem-cefotaxime, imipenem-amikacin, amikacin-cefotaxime, and amikacin-TMP-SMX.

The antibiotics used in this study and their sources were as follows: imipenem, Merck & Co., Inc., Rahway, N.J.; amikacin, Bristol Laboratories, Syracuse, N.Y.; TMP-SMX, Burroughs-Wellcome Co., Research Triangle Park, N.C.; and cefotaxime, Hoechst-Roussel Pharmaceuticals, Inc., Somerville, N.J.

A total of 26 clinical isolates of *N. asteroides* were identified by standard criteria and have been previously described (3). All isolates were maintained on Sabouraud agar slants. A loopful of each isolate was placed in Mueller-Hinton broth containing sterile glass beads (1 mm in diameter). The tubes were incubated at 37°C in a shaker bath and vortexed frequently for 3 days,

after which time 1 ml of homogeneous suspension was subcultured into 5 ml of Mueller-Hinton broth containing glass beads. These suspensions were incubated as described before for approximately 72 h (3).

After this incubation period, colony counts were performed; each isolate was in the range of  $10^6$  to  $10^7$  CFU/ml. A Steers replicating apparatus was used which delivered 0.002 ml per inoculum spot on Mueller-Hinton agar. The final inoculum was  $10^3$  to  $10^4$  CFU/ml. The agar plates contained antibiotics in serial twofold dilutions with concentration ranges as follows: imipenem, 16 to 0.0075 µg/ml; TMP-SMX (1:20), 4-80 to 0.015-0.3 µg/ml; cefotaxime, 16 to 0.0075 µg/ml; and amikacin, 8 to 0.03 µg/ml. The MIC was defined as the lowest antibiotic concentration suppressing all growth at 48 h of incubation at 37°C.

Synergy was present by the agar dilution method when there was a fourfold or greater reduction in the MICs of both antibiotics. A reduction of less than fourfold in the MICs of both antibiotics was considered additive. Indifference was found when neither drug exhibited a decrease in MIC, and an increase in the MIC was considered antagonism.

The susceptibilities of 26 *N. asteroides* isolates to imipenem, amikacin, TMP-SMX, and cefotaxime are shown in Table 1. These numbers represent the mean values of results obtained from five separate synergy tests with different antibiotic combinations.

Testing for synergy by the agar dilution technique is based on inhibitory rather than bactericidal endpoints. The antibiotic combinations tested for synergy are shown in Table 2. Of the currently available agents, amikacin and TMP-SMX are the most active in combination with

TABLE 1. Comparative activity of antimicrobial agents against 26 *N. asteroides* strains

Antibiotic	MIC ( $\mu\text{g/ml}$ ) <sup>a</sup>		
	Range	50%	90%
Imipenem	0.06–2.6	0.58	1.66
Cefotaxime	0.37–>16	3	>16
Amikacin	0.38–3.6	0.92	3.3
TMP-SMX (1:20)	0.09–1.9–5–100	0.6–12.5	3–60

<sup>a</sup> Results are shown as mean values of multiple tests.

each other. Imipenem in combination with TMP-SMX or cefotaxime also showed a very high percentage of synergy in this test system. The combinations of imipenem-cefotaxime and amikacin-cefotaxime did not show the same degree of activity, with most of the combinations showing an additive effect. The fractional inhibitory concentration was  $\leq 0.05$  in all combinations that resulted in synergy. There was no antagonism found in any of the combinations tested.

Susceptibility testing of *N. asteroides* has been hampered by the lack of standardized testing because of the many variables encountered in growing the organisms uniformly. Also, there has been poor correlation between antibiotic susceptibility in vitro and antibiotic efficacy in experimental models (1), owing in part, presumably, to an inoculum effect (5). This is particularly true in the case of the sulfonamides. There is better correlation, however, when lower inocula are used (4).

Several new  $\beta$ -lactam compounds have been developed which are active against *N. asteroides*. If sulfonamide therapy alone for nocardial infections either cannot be tolerated because of side effects or proves ineffective, in vitro susceptibility testing in which these  $\beta$ -lactams are used with a sulfonamide or other agents such as amikacin, ampicillin, and erythromycin may show increased activity. Several authors have had success using combination antimicrobial therapy (2, 4) for nocardial infections.

Several studies have confirmed that imipenem and amikacin are the two most active agents on a weight basis against *N. asteroides*. The combination of these agents with cefotaxime and TMP-SMX were synergistic in most cases (Table 2). The most effective combination in the

TABLE 2. Antibiotic combinations against 26 *N. asteroides* isolates

Antibiotic combination	No. of isolates showing %:			
	Synergy	Additive	Indifference	Antagonism
Imipenem–TMP-SMX	80	20	0	0
Imipenem–cefotaxime <sup>a</sup>	92	8	0	0
Imipenem–Amikacin	5	75	20	0
Amikacin–cefotaxime <sup>b</sup>	26	66	8	0
Amikacin–TMP-SMX	83	17	0	0

<sup>a</sup> Thirteen strains tested.

<sup>b</sup> Fifteen strains tested.

present study was imipenem and cefotaxime, which resulted in synergy in 92% of tests. These are two  $\beta$ -lactam compounds, and since their mechanisms of action are similar, the results are surprising. They may act at different sites of cell wall synthesis or bind to different penicillin-binding proteins. The imipenem-amikacin combination, which represents classes of antibiotics frequently synergistic against several bacterial species, showed a predominantly additive effect.

Inhibitory endpoints were assessed in determining synergy, and in vivo data may not correlate with clinical effectiveness. Further investigation with an experimental model is warranted.

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